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PROMOTION AND INHIBITION: TWIN THEMES OF PHYSIOLOGY*

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Many of us, whether zoologists or botanists, whether we are studying physiology or genetics, growth or death, working in the laboratory or in the great outdoors, are mainly measuring the rate of something and interpreting these rate measurements to advance the front of biology. Not only are we measuring rates, but we are altering them—by temperature perhaps, or pressure, or nutrition, or genetic change, or a hormone—and from the acceleration or inhibition we make deductions about our subject.

Some fortunate geneticists may claim to be exempted from this generalization, since in their cultures they may be concerned simply with growth or no growth, ability or failure to synthesize a metabolite—that is, rates infinitely low or infinitely high—yet in a surprising number of these cases it does develop that the matter is not so black and white as it seemed, and at another temperature or with some other change of conditions the missing process does go at a measurable rate.

Some morphologists may think that they are exempt from the study of biological rates, but the fact is that morphology is essentially the expression of *balance* between reaction rates. A river broadens out into lakes in flat country but narrows into a torrent on the hillside; its morphology is thus a direct expression of the *rates of flow* of water in and out. Similarly a meristem is a mass of small cells, because its cells divide faster than they enlarge; pith, or storage tissue, on the other hand, (large, thin-walled cells showing few mitotic figures) results when cells enlarge faster than they divide. We can depict this in morphological terms, that is, in photographs, or we can describe it in physiological terms, that is, in terms of *relative rates*. Such considerations, obvious in themselves, remind us that even when we think otherwise we may actually be studying the acceleration or inhibition of biological rates.

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Inhibition is a universal phenomenon throughout the biological world. All sorts of substances and circumstances cause inhibitions, and all sorts of processes are subject to them. Freud was the first to show that without our personal inhibitions we would be odd and uncharacteristic creatures. By what physical mechanism these are exerted, it is as yet too soon to say. A less delicate, but still elusive example is offered by the social hormones of insects. In a hive of bees, when the queen is removed, the workers begin to produce eggs. Apparently under normal conditions egg development in worker bees is inhibited by traces of a substance produced on the outside of the abdomen of the queen, and distributed to the workers through the food (Butler, 1954). A dead queen replaced in the hive will re-establish the inhibition of egg development, at least for a time. Such dominance exercised by an excretion is doubtless limited to the insects; in small boys, dominance, with inhibition of too active competition, is exercised by physical prowess; in plants dominance of one bud over another is exercised by an internal secretion.

OPTIMUM PHENOMENA WITH A SINGLE COMPOUND

This last point brings us to a major theme, namely that inhibition and stimulation are often caused by the same substance. If a substance promotes or stimulates a process, then it commonly inhibits it in higher concentrations. The effect of dinitrophenol on the respiration of yeast is a classical example. Similarly, if a substance typically inhibits a process it commonly (not always) stimulates it at sufficiently low concentrations.

The vertebrate heart is slowed by application of acetylcholine, but if precautions are taken to lower the internal level of acetylcholine, then low applied concentrations excite it (see Welsh, 1955). Antagonists of acetylcholine may produce excitation of the Venus heart, suggesting that its normal content of the hormone is on the verge of the inhibitory level. Adrenalin shows a similar action on the salivary glands. Numerous depressant drugs are well known to cause excitation when in low concentrations. The action of auxins on plants presents some remarkably clear cases of these changes of sign.

Auxins, as is well known, promote the growth of shoots, stimulating both cell division and cell enlargement, but they inhibit the growth of roots. Inhibition is obtained at quite low concentrations (about 10^{-8} molar) with all roots tested. Yet at the lowest level of concentration, root growth is usually somewhat promoted. The effect is small, but has been observed in roots of many different plants, and particularly strongly in isolated sections of roots.

Lateral buds are inhibited by the very same auxin concentration which stimulates the growth of the axis on which these buds lie. Thus they remain inhibited so long as the terminal bud goes on producing auxin. As the terminal bud grows away from them the auxin supply becomes weakened by distance, so that eventually laterals far down the stem begin to grow out.

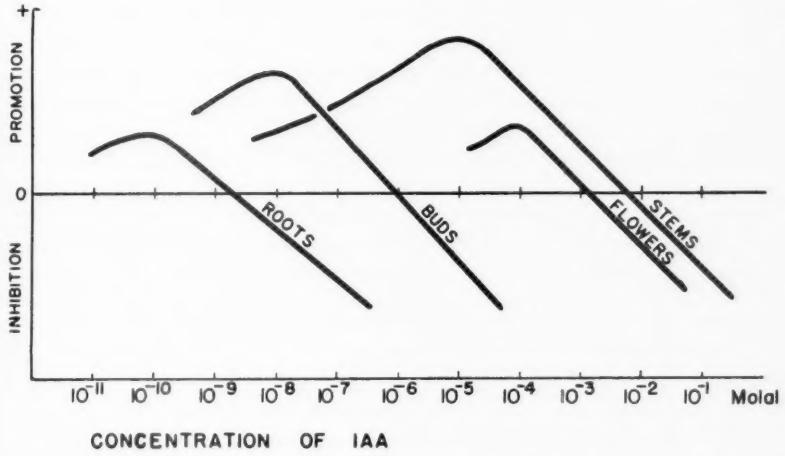


FIGURE 1. The effect of indole-acetic acid (IAA) on the growth of different organs (diagrammatic). From Leopold and Thimann, 1949.

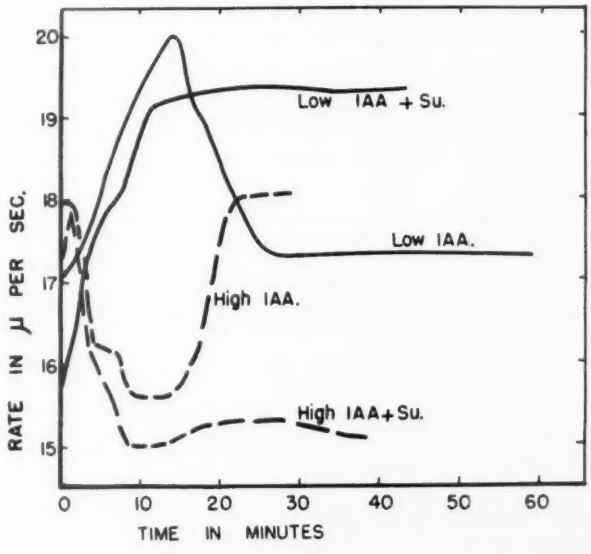


FIGURE 2. Changes in the rate of protoplasmic streaming in epidermal cells of the Avena coleoptile. Auxin (IAA) is added at time zero in each case, and the rates of streaming determined every 1–3 minutes thereafter. Note that IAA alone causes an increase or decrease in rate which soon returns to normal, while IAA and sucrose (Su) causes an increase or decrease which is maintained. Redrawn from Sweeney and Thimann, 1938.

This is an example of *weakening of inhibition with increasing age*, but it obviously has a simple explanation, namely that access of the inhibitor falls off. (True change of inhibition with aging does occur also, however, and will be mentioned below.) The relations between growth and auxin concentration for different organs are expressed in figure 1.

A nice example of promotion and inhibition in the same cell is given by the effect of auxin on protoplasmic streaming in epidermal cells of Avena coleoptiles. At low concentrations, streaming is markedly accelerated, but at about 5.10^{-5} molar and above the rate is decreased, and in the presence of sugar it may remain depressed for hours (figure 2).

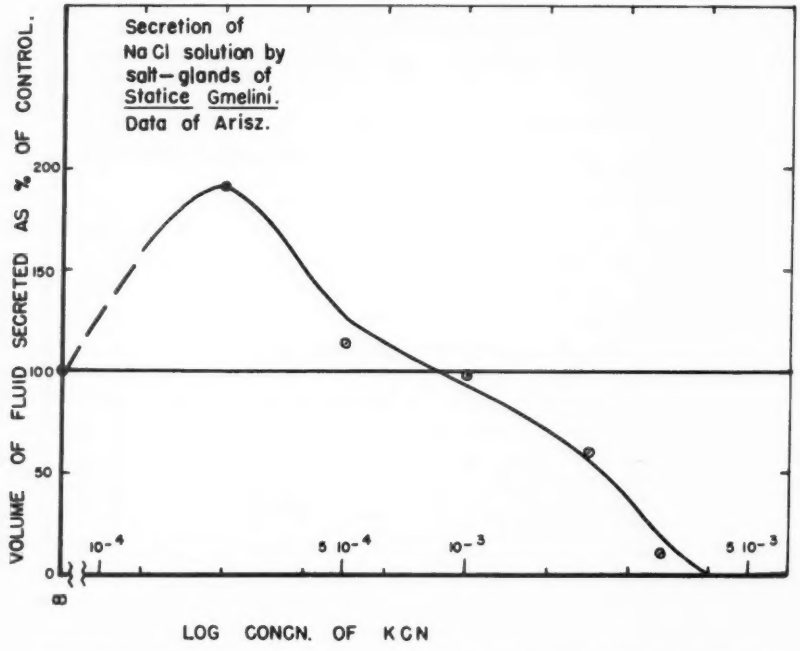


FIGURE 3. Inhibition and promotion of salt secretion by salt glands of Statice, caused by potassium cyanide. Plotted from data of Arisz, Camphius, Heikens and van Tooren (1955).

Where such clear optima are concerned it is hard to say whether we are dealing primarily with a stimulating substance which inhibits at high concentrations, or with an inhibiting substance which stimulates at low concentrations. One thinks of auxin as a stimulating substance because growth stimulation is its most typical quality. But consider typical inhibitors of enzymes. Cyanide inhibits cytochrome oxidase, and as a result it inhibits respiration and processes dependent on respiration, but in low concentrations it promotes some of these. A recent striking example is its

large promotion of salt excretion in the glands of Statice (Limonium) plants, studied by Arisz et al. (1955). Concentrations of cyanide about five times the promoting level inhibit in the normal way (figure 3), and these are in the range where respiratory inhibition would be expected. Oxygen itself shows a marked optimum curve for growth of shoots and coleoptiles, air being clearly too high, and the maximum growth rate occurs at 3–8 percent O_2 (Ranson and Parija, 1955). This reaction is apparently due to effects on the auxin system.

Optimum phenomena used to be referred to in the German literature as the Arndt-Schulz law, having been formulated by Arndt in 1882 from experiments on animals, and by Schulz in 1888 from studies on yeast fermentation. The "law" can be paraphrased: Every poison causes either a reduction or an increase in physiological performance, corresponding to the larger or smaller "intensity" of its stimulus, that is, its concentration. Of course, there are many exceptions. Many enzyme inhibitors show no promotion at all, particularly when acting on isolated enzyme systems. On the other hand in one case a *double* change of sign has been reported; aspartic acid in very low concentration promotes growth of certain tissue cultures; higher concentrations inhibit, but very high concentrations promote again (Riker and Gutsche, 1948).

In a recent discussion of the numerous growth inhibitors for bacteria (Thimann, 1955), these substances were divided into six types: (1) substances forming complexes with the heavy metals of enzymes, for example, HCN, CO, 8-hydroxy-quinoline, α-α-dipyridyl; (2) sulfhydryl reagents; iodoacetate, heavy metals, especially in organic form, arsenite, mustard gases, unsaturated lactones, and probably the oxidizing radicals formed by X-rays and other radiation; (3) soaps, phenols, detergents and similar compounds which act by being adsorbed at surfaces and membranes; (4) dyes, especially active on Gram-positive bacteria and fungi; (5) substances structurally resembling essential metabolites—an enormous group, ranging from malonic acid through sulfanilamide to the antivitamins and recently antiauxins; with the general characteristic that the inhibition they cause can usually be relieved by excess of the normal metabolite and hence is labeled "competitive"; (6) the inhibitors from natural sources, like the antibiotics, whose mode of action is generally unknown.

Group 2, the sulfhydryl reagents, offer some very marked reversals of effect. For example, as far back as 1927 Niethammer showed that silver, lead, and other metals promote the growth of Aspergillus very considerably at low concentrations, while they inhibit at high. Inhibition usually sets in at $\frac{M}{1000}$ to $\frac{M}{10{,}000}$, depending on the metal used; promotion is optimal at about 1/10 this level.

The effect of iodoacetate on growth of Avena coleoptile sections is also striking; low concentrations can increase growth by as much as 60 percent (figure 4). Note, however, that promotion depends on the age of the cole-

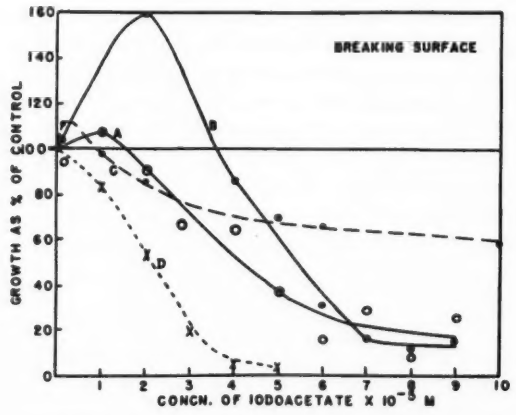


FIGURE 4. Inhibition and promotion of growth of isolated sections cut from Avena coleoptiles of different ages, caused by iodoacetate. Curve A 74 hrs., B 64–66 hrs., C 54 hrs., D 96 hrs. From Thimann and Bonner, 1948.

optile. Very old coleoptiles show only inhibition, while young coleoptiles are generally insensitive and show little promotion or inhibition. Organic mercurials show similar effects but the extent of growth promotion is smaller. Growth promotions have been recorded as a result of X-radiation too.

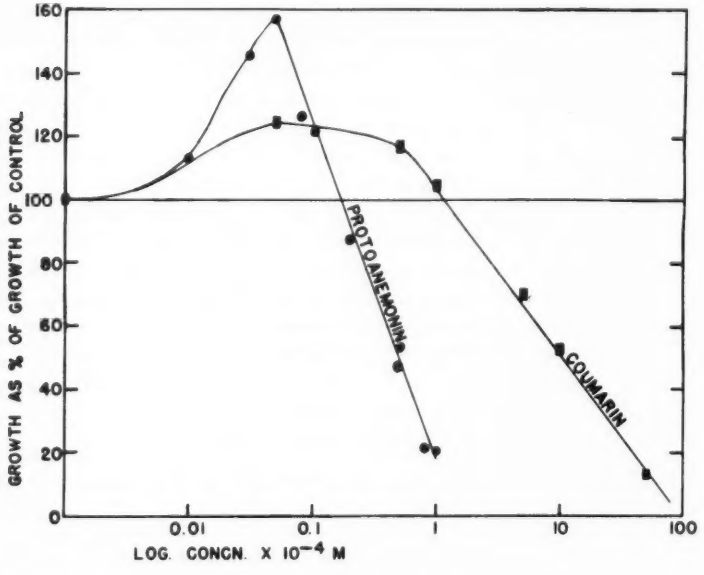
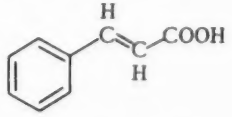


FIGURE 5. Inhibition and promotion of growth of coleoptile sections caused by two unsaturated lactones. From Thimann and Bonner, 1949a.

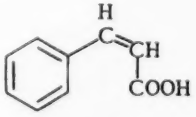
These effects make one wonder whether perhaps plants contain a natural growth inhibiting system, which is excessively sensitive to SH reagents. If such a system exists, then normal growth would be due to a balance between it and the growth-promoting hormone system.

Still more striking are the effects of unsaturated lactones such as coumarin. These compounds also react with SH groups, as is shown by the ease with which the growth inhibition they cause can be reversed by BAL (2,3-dimercaptopropanol-1). Their action on the coleoptile is characterized by massive growth promotion at a wide range of low concentrations (figure 5). In these cases, one is at a loss to decide whether these compounds are really to be regarded as growth promoters or growth inhibitors.

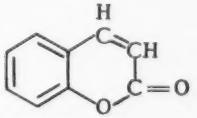
I suspect that at least some of these unsaturated lactones represent a special case. From the study of the auxin activity of many compounds it has become clear that the structural requirements which a compound must possess to have activity include an unsaturated ring, a sidechain bearing an acid group, and a certain spatial relation between these two. (Certain other planar structures may take the place of a ring.) In acids with an unsaturated sidechain, this spatial relation is shown in the fact that the *cis* isomer is active while the *trans* isomer is not. *Trans*-cinnamic acid and some of its derivatives, *trans*-naphthaleneacrylic acid and *trans*-tetralidene-1-acetic acid are inactive—their *cis* enantiomorphs all show varying degrees of activity (see Veldstra, 1953). Now a lactone is of necessity derived from a *cis* acid (by condensation with a hydroxy-group) so it is an unsaturated ring compound of *cis* configuration. It is, therefore, suggested that the *growth promoting* part of the action curve of these substances is due to their close similarity to the auxin structure. Thus these, and perhaps some other compounds too, are to be considered as simultaneously *both* promoters and inhibitors.



Trans-(inactive)



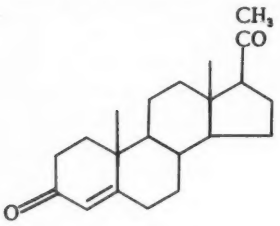
Cis-(active)



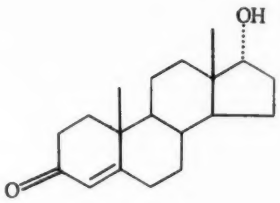
Lactone

Such a combination of opposing properties is not unknown in other fields. An example is given by certain thyroxin analogues, the nitro-ethers of N-acetyl diiodotyrosine. These compounds have weak thyroxine activity in tadpoles, promoting the metamorphosis into frogs, but they also antagonize the effect of natural thyroxine, (Woolley, 1946). In the field of sex hormones the strange case of pregneninolone must be remembered; this compound possesses progestational, estrogenic and androgenic activities, in spite of the fact that the first two are clearly opposed to one another in their action on the uterus, while the second and third are certainly opposed in their general activity (see Courrier, 1950). Testosterone also has the same three activities. Here the structural requirements for activity differ

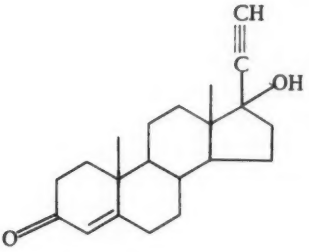
so little between the three hormonal types that one can well imagine the same compound simultaneously satisfying the requirements for all three.



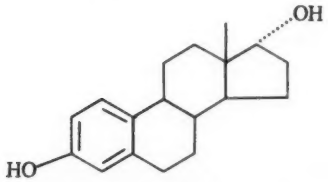
Progesterone
(Gestagenic)



Testosterone
(Androgenic)



Pregneninol-17-one-3



Estradiol
(Estrogenic)

INTERCHANGE OF PROMOTION AND INHIBITION: BIOLOGICAL BALANCE

Another characteristic of inhibition and promotion is that the relation between the two is easily altered. We have seen examples of its dependence on age. It is also changed by *duration of experiment.* The most familiar example of this is the development of resistance. Bacteria treated with an antibiotic mutate to a resistant or independent form, which is able to grow rapidly in the virtually uninhabited medium. The result is that very soon the inhibition has disappeared and the organisms (carrying a single mutation but otherwise recognizable as the original organisms) appear as flourishing as ever. A particularly nice example is furnished by the housefly treated with DDT. This animal develops resistance by the simple expe-

dient of producing a dehydrochlorinase which splits HCl out of the DDT and thereby renders it harmless. Fortunately, not many insects are able to do this, or our control problems would be seriously multiplied.

But inhibitions of growth change into promotions sometimes without the intervention of mutant forms. Aspergillus, inhibited in growth by a fungicide, 2,2′-methylene-bis(4-chlorophenol), shows recovery after some days. By the time the cultures are 15 days old the fungicide is clearly promoting growth in the lower concentrations. Such recovery only occurs when the nutrient is supplied at high concentration (table 1). This promotion could, of course, be due to a greater inhibition of respiration than of growth, so that after a while more of the substrate becomes available for growth in presence of the fungicide than in the control. This would only be likely to occur when the substrate level was high enough so that it would not have been exhausted in the early days of the growth.

There are, indeed, numerous cases where inhibition or promotion results according to the substrate or energy source supplied. Niethammer (1927)

TABLE 1.

INHIBITION AND GROWTH PROMOTION OF *ASPERGILLUS NIGER* BY A BIS-CHLOROPHENOL. (DATA OF WATKINS AND CLEMME, 1948).

	Mycelial weight in 15 days		
Concentration of Dextrose:	1%	4%	8%
Control	210	390	390
With fungicide, 30 p.p.m.	110	380	535
As percent of control	52	97	137

found that manganese, lead and an organic mercury compound only stimulated the growth of *Aspergillus niger* when ample sugar was supplied. When the sugar was more nearly in limiting concentration these metals only inhibited growth, at all concentrations (*cf.* the fungicide above). A curious example arose recently in a study of the action of cobalt on the growth of plant sections. Miller (1954) had reported that cobalt alone promotes elongation of pea stem segments only weakly but that if sugar is added the promotion is much enhanced. It now develops, especially with Avena coleoptiles, that the cobalt effect has a quite different character with different substrates (Thimann, 1956). With sugar there is a clear optimum, along with inhibition at high cobalt concentrations (probably due to interaction with a sulfhydryl enzyme). With malate no such optimum is found, but the highest cobalt levels continue to increase the growth, though the whole effect is much smaller than with sugar. With acetate plus sugar, on the other hand, there is practically no growth promotion, and almost all cobalt concentrations inhibit strongly (Figure 6). This behavior may best be inter-

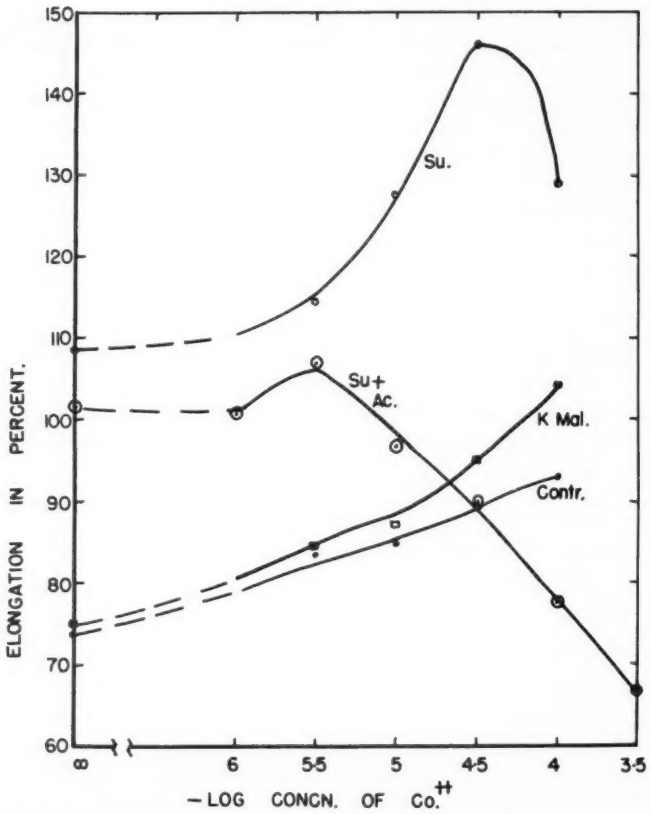


FIGURE 6. Inhibition and promotion of growth of coleoptile sections by Cobalt as function of the carbon source. Su = sucrose 2%, (0.06M); Ac = potassium acetate 2.10^{-3}M plus Su 2%; KMal = potassium malate 10^{-3}M; Contr = control. All solutions contained auxin (IAA) at a concn. of 3 mg. per liter. Redrawn from Thimann, 1956.

preted in terms of competition between different metabolic paths which are affected in different ways by stimulation of the same enzyme.

In the intact cell (or tissue) there are likely to be a number of metabolic systems influencing any one process, some promoting, some inhibiting or delaying. The action of any given inhibitor or promoter, even if it seems as simple as that of cyanide, must therefore be viewed in terms of altering a balance between opposing forces.

We can get an idea of how the cell achieves such balances from the well worked-out effects of dinitrophenol (DNP) and its congeners. Classically, these compounds stimulate respiration in Arbacia eggs, yeast and animal tissues, but inhibit it at high concentrations. The explanation now gen-

erally accepted is that DNP acts by inhibiting the phosphorylation that is normally linked to respiration. The oxidation of every molecule of hexose leads to the formation of 30 to 40 molecules of ATP by the phosphorylation of the diphosphate, ADP. As a result there is normally an overproduction of ATP by respiration, and a permanent shortage of ADP. However, a few critical reactions are dependent on the presence of ADP as acceptor of phosphate residues; the respiration rate is therefore limited by ADP (or other phosphate acceptors). Hence by inhibiting phosphorylation, nitro-

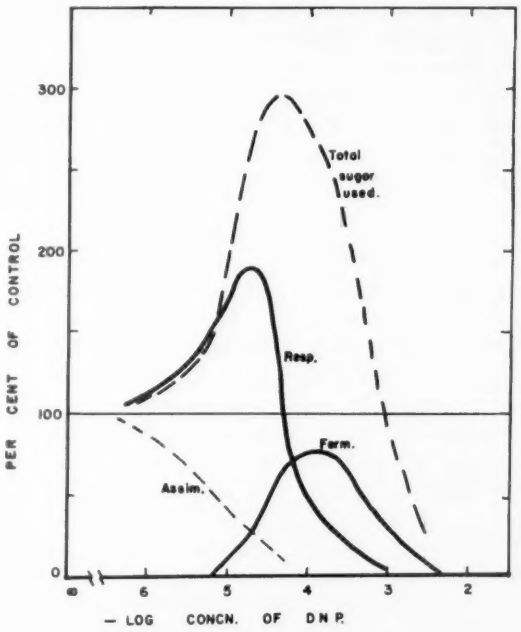


FIGURE 7. The effects of 2,4-dinitrophenol (DNP) on sugar metabolism by a baker's yeast. The rates of fermentation are expressed as percentage of the CO_2 production of the control. Replotted from figures of Simon (1953).

phenols make ADP available and respiration can increase. At really high DNP concentrations, so much ADP becomes present that even fermentation can start. Now a second acceleration is superimposed, for fermentation (when it does begin) uses up sugar even faster than does respiration (figure 7). Thus DNP causes a tremendous increase in sugar consumption. But the prevention of phosphorylation means that, at all concentrations, synthetic or assimilatory processes (*assim.* in fig. 7) are inhibited. Only at very high concentrations are fermentation and sugar usage inhibited, presumably because of a too powerful interference with phosphorylations.

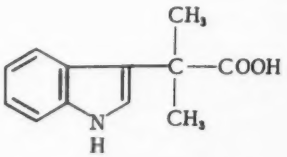
The action of thyroxin has of course been compared to that of nitrophenol; it increases respiratory rate apparently by making phosphate acceptors available, as both Martius and Lipmann have shown. It thus decreases syntheses of many kinds, including that of fat. Yet low, normal thyroxin concentrations are essential for normal activity, and they actually stimulate those syntheses which are dependent on ATP. The effect of thyroxin on syntheses must, therefore, be represented by an optimum curve. To explain this, Hoch and Lipmann have suggested (1953) that if the linkage between respiration and phosphorylation is very tight then all the hydrogen of organic compounds must be oxidized to H_2O, yielding ATP, whereas in order to achieve normal syntheses some hydrogen must be available for chemical reductions. In other words, too tight a linkage is no more desirable than too loose a one, but both hydrogen and ATP (may we say both matter and energy?) must be available. Put in another way, a small decrease in the energy yield, by making hydrogen available for reductions, actually causes an increase in the syntheses.

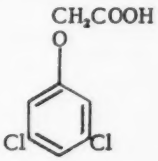
LIMITING FACTORS

The promotions of respiration discussed above raise some tantalizing questions about limiting factors. Most plant and animal tissues, if treated with glucose, increase their rate of respiration. We can therefore conclude that respiratory rate is being limited by the glucose, or shall we say in general by the SUBSTRATE. But now respiration is very sensitive to cyanide, or to CO in the dark. These agents inhibit cytochrome oxidase, the terminal oxygen-activating enzyme. From this we deduce (contrariwise) that the factor limiting respiration must be the OXIDASE. Worse, however, we have just seen that nitrophenol increases respiration by making phosphate acceptors available, from which we deduced that respiration is limited by the PHOSPHATE ACCEPTORS, and that the substrate must be in excess. In the case of yeast all three experiments have actually been done on the same organism. It certainly looks as though there must be something wrong with our reasoning. As far as cyanide is concerned we can perhaps extricate ourselves from the tangle by making the assumption that the oxidase was not really the limiting factor, but that doses of cyanide too low to show any inhibition already combine with the oxidase, and only as the cyanide dosage is increased is the point finally reached at which the amount of functioning oxidase has been cut down to where it does become the limiting factor. This could of course be tested. But as for the other two, limitation by substrate and by phosphate acceptor, they seem virtually incompatible. One is reminded of the amorous poet's lament, "how happy could I be with either, Were t'other fair charmer away."

Inhibitions of another sort, but based also on competition, have recently come into prominence in plant physiology, for some of the substances which structurally resemble auxins act antagonistically to auxin, inhibiting

growth in shoots and increasing it in roots. Among the most effective compounds for increasing root growth are indoleisobutyric acid and 3,5 D:





It is not my purpose to review this extensive field here; it is much too long and too technical, but one or two comments may be worth making. One is that in this, or any other hormone-antihormone system, it is essential to keep in mind the concept of limiting factors already mentioned. Kinetic analyses have been used to show that there is the same strict competition between auxin and antiauxin in the growth of test plants as, for example, between succinate and malonate in the extracted succinic dehydrogenase enzyme. The demonstration essentially depends on the fact that the higher the concentration of auxin the less marked is the inhibiting action of the antiauxin. But now consider what are the limiting factors in growth. At low auxin concentrations, it is presumable that auxin is the limiting factor, and anything which interferes with its action would have maximal effect. At high auxin concentrations the growth rate is more nearly limited by the energy supply, or the rate of entry of water through the cell membranes, etc., so that an auxin antagonist would have minimal effect. Thus the reduced effectiveness of the antagonist in presence of high suxin need not be due to its actually being *displaced* by auxin, but only to its acting in a system where auxin is not the limiting factor. In this way, the competitive nature of the inhibition may be only apparent. The complementary case is the one in which an inhibitor acts only on the energy source, and therefore should have a much greater effect at high auxin levels (where the energy source would be limiting) than at low ones. Exactly this behavior has in fact been demonstrated for growth inhibition by arsenite (Thimann and Bonner, 1949b).

In roots the relationships are very complex. Auxin alone inhibits the elongation. Antiauxins promote root elongation, presumably by antagonizing the auxin. But a second antiauxin added at the same time appears to antagonize the first, decreasing the growth again (Hansen, 1954). It seems almost impossible to explain this on the ground that auxin and the antiauxins function at the same site. Burström has suggested that perhaps the antiauxins act also on the system causing auxin uptake. If they inhibited auxin uptake, of course, they would stimulate growth in added auxin, and if they inhibited each other's uptake they would antagonize each other. Perhaps it is suggestive that triiodobenzoic acid—an antiauxin by many standards—has recently been shown to inhibit the transport of auxin (Kuse,

1953; Hay, 1955). Transport from cell to cell certainly has much in common with uptake which is transport from solution to cell. Furthermore Reinhold (1954) has shown in stem tissue that one auxin (2,4-D) does inhibit the uptake of another (indoleacetic acid). One is tempted to speculate that biological antagonisms, at any rate those between externally applied hormones, may in many cases be exerted on the absorption rather on the hormone-controlled process itself. This will certainly bear examining in the near future.

BASIS OF THE OPTIMUM CURVE

It only remains to propose an explanation for the optimum curve in at least the special case of auxin. One explanation has already been given, based on the idea that the auxin molecule causes growth by combining with two other molecules, namely an enzyme and a substrate, which it thus brings together (Skoog *et al.*, 1942). If the auxin is in excess, the enzyme molecules combine with some of them while the substrate molecules combine with others. Thus the enzyme and substrate are not brought together but held apart. This explanation is certainly possible, but the following one is believed to be more general in nature.

Let us suppose that the auxin does not cause growth by combining directly with its receptor molecule or enzyme, but only after conversion to a modified form. (Such a form might be indoleacetyl phosphate or indoleacetyl co-enzyme A, to mention only two possibilities.) If the auxin is in excess, much of it cannot be converted to this modified form because the *converting system* (phosphokinase or other enzyme) is saturated. Hence the unconverted, inactive auxin is in competition with the active converted form, and may thus inhibit the growth reaction, just as glyceraldehyde inhibits the fermentation of phosphoglyceraldehyde. In this way the different optimum curves for different plant organs (Figure 1) could be explained by different levels of a single enzyme, namely the enzyme of the *converting system.* This theory may prove a very useful one, since it is capable of wide application, and could explain the optimum curves given by a great variety of vitamins, hormones and drugs.

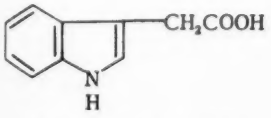
RELIEF OF INHIBITION

Where inhibition is due to combination with a sulfhydryl enzyme or co-enzyme it can sometimes be prevented or relieved by protecting the SH group. This was shown long ago by Hopkins when he protected succinic dehydrogenase against maleic acid by adding glutathione. In growth experiments, plant sections could be protected against the coumarin inhibition by administering the sulfur compound BAL, as mentioned above. If in a similar way we could protect against the inhibition by high auxin concentrations this would give a definite lead to the site of action of auxin, a key botanical problem and one which indeed remains unsolved for nearly all

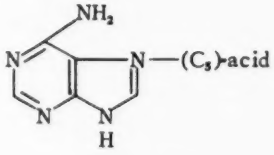
hormones. Two interesting advances have been made in this direction recently.

Growth of the fungus *Nectria galligena* is inhibited by high auxin concentrations (20 to 100 mg. of indoleacetic acid per liter) and this inhibition is prevented or relieved by an extract of cotton or better still by biotin (Nysterakis, 1954). As little as 1/10γ biotin counteracts 100 mg. auxin.

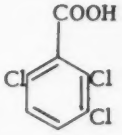
The inhibition of bud development by auxin has been especially studied in growing tissue cultures where auxin inhibits bud *formation*, a similar phenomenon but one more open to experiment. Here the inhibition can be largely relieved by adenine (Skoog and Tsui, 1951, Miller and Skoog, 1953). Phosphate increases the action of adenine and adenosine is also effective. In presence of adenine, other purines and pyrimidines increase the effect. 6-Furfuryl adenine is much more effective than adenine. From this and other work it has been concluded that the inhibiting action of auxin is exerted on nucleic acid formation. Much confirmatory evidence points in the same direction. The morphology of tissue cultures producing buds and roots depends in large part on the auxin-adenine balance and there are now several recorded interactions between auxin and adenine or other purines. After considering structural relations between auxins and antiauxins it is tempting to speculate further on the purines. No one seems to have noticed the very suggestive similarity of the formulae:



Indoleacetic Acid (IAA)

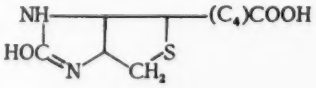


Adenosine



2,3,6-Trichlorobenzoic acid

Is it too far-fetched to suggest that such compounds might react at the same enzyme system? The formulae are certainly not further apart than IAA and some synthetic auxins, for example, trichlorobenzoic acid, which is as active as IAA. And could one perhaps, even more fancifully, implicate biotin:



Biotin

I believe these possibilities should be taken seriously.

SYNERGISM

One curious phenomenon which often goes along with inhibition is synergism. Some auxin-like compounds increase the effectiveness of low con-

centrations of auxin. Triiodobenzoic acid is perhaps the best example, causing synergism in inhibiting the growth of roots and in promoting growth of coleoptiles and especially curvature of pea stems. Synergists frequently have all but one of the structural features of the molecule necessary for auxin activity, as was pointed out by Went (1949). Similar phenomena are known with the steroid hormones, particularly the action of progesterone in increasing the response of the uterus to estrogens (see Courrier 1950). How are they to be explained? For if the hormone-like substance combines with the same locus as the hormone it can only antagonize. In the special case of auxins it is interesting to note one point: synergism is larger and easier to obtain in the pea test than in growth of Avena coleoptiles. And it has long been known that the pea test is less specific for synthetic auxins than tests using Avena coleoptiles; a wider range of substances shows activity in peas, and the activity of a given synthetic compound is almost always relatively higher in peas. With this preliminary a suggestion can be made about auxin synergism. For if the requirements for activity

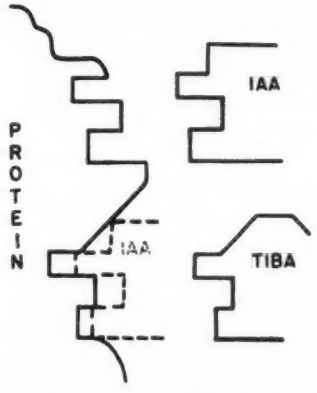


FIGURE 8. Synergism. The enzyme (left) is depicted as containing combining sites of slightly different morphology. The auxin (IAA) and synergist (TIBA) combine preferentially each at a different site.

are less stringent in one plant than in another, there must be a difference between the combining sites in the two plants. For instance, the combining sites in the pea plant might be more flexible. But there is another and more interesting possibility: the combining sites might be more *varied*. There seems no necessity to assume that all combining sites in one plant are the same. Suppose there are a family of them, closely similar but not identical. Some fit the auxin molecule exactly, and combine with auxin rapidly and with high affinity. Some fit less well and hence combine more slowly and/or with lower affinity (Figure 8). But they fit better with another substance similar to, though not identical with, auxin. Hence this other substance,

for example, triiodobenzoic acid, will combine preferentially with these less closefitting sites and as a result it will displace the auxin from them. Auxin, on the other hand, will displace the triiodobenzoic acid from the sites where the auxin molecule fits best. As a result, in presence of the synergist more auxin molecules will become combined at their most effective sites; the auxin molecules will hence function more effectively and more growth will ensue. Of course if the synergist concentration is too high it will begin to interfere with the combination of auxin at its effective sites. Thus the synergists all inhibit growth at high concentrations.

Considerations of limiting factors are of interest here too. It is evident that the action I have described would only be effective when the auxin is in low, and limiting, concentration. At high auxin concentrations all the effective centers are occupied and the synergist can do no more. A nice example is seen in the rooting of certain cuttings, in which indole, which is quite inactive in itself, synergizes with indoleacetic acid. At low indoleacetic concentrations, 2 to 18 ppm., the indole about doubles the number of roots; at high concentrations (50 to 100 ppm.) it scarcely increases them at all (van Raalte, 1951). Yet this is certainly not to be attributed to competition; indeed almost the reverse. The root-forming ability is simply limited, at high auxin concentrations, by other factors. So the evidence which is often taken as showing strictly "competitive" action may not really show this, but may be a natural consequence of the interaction of a group of limiting factors in controlling a multifactor process.

CONCLUSION

Thoughts and speculations of this kind are, one hopes, occasionally justified if they assist in the visualizing of a number of apparently unrelated phenomena as a *group*, with a common pattern, or if they help one to discern a biological principle in a pile of experimental data.

The principle that inhibition is often or usually accompanied by a change in the balance between opposing reactions seems to emerge with clarity: promotion of one reaction can be due to inhibition of another and vice versa, through the resulting removal of limiting factors, or the accumulation of reagents which previously were rate-limiting. Overall processes like cell enlargement or the synthesis of complex compounds appear to be commonly subject to multiple control. It is this, probably, which gives the cell its enormous flexibility, and helps the organism to survive under a variety of unfavorable conditions. It is this which lies at the heart of the cancer problem, where a small and subtle change in the balance between growth promotion and inhibition leads to a long-continued overgrowth. Perhaps indeed it is this interaction and balance between multiple systems that is the real distinguishing characteristic of living organisms.

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INTERNAL FACTORS CONTROLLING CELL DIFFERENTIATION IN THE FLOWERING PLANTS*

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The idea that one chemical, or perhaps one physical process, normally controls the differentiation of a given cell type is common coin in biology. But though the idea is common, substantial evidence in support of it is rather rare. This research was started in the hope of finding extensive and quantitative evidence linking a naturally occurring substance to the differentiation of a given plant cell type.

To make the job easier, the substance was picked first and then a search was made for a cell-type to go with it. Auxin, the plant growth hormone, has two useful characteristics: (1) it has been reported by many researchers to show strictly polar movement and (2) amounts of auxin can be measured in an excellent, quantitative bioassay called the "Avena test" (cf. Went and Thimann, 1937). Since the reported strictly polar movement of auxin (from the original tip to the original base, irrespective of gravity) is so unusual, I scoured the botanical literature for reports of some cell-type which showed a matching polar differentiation. Such a cell-type was found in xylem, the cells which serve as channels for the transport of water and salts. Xylem cells occur in strands which run up and down the stem and out into the leaves. If one of these strands of xylem cells is cut, a number of investigators have reported that the xylem strand regenerates, but only toward the bottom of the stem. The new xylem cells differentiate by quite direct transformation of the unspecialized cells called parenchyma. In this transformation, a new cell wall is laid down inside the old one, and often portions of the old wall are dissolved to form holes directly connecting the lumen of one new xylem cell with that of the next.

QUALITATIVE RESULTS

By cutting off leaves and pieces of stem, using agar blocks to collect auxin from their cut surfaces, it was found that little or no auxin was produced by the stem, but large amounts were produced by the leaves—

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particularly the leaves which were growing the fastest (fig. 1). This was expected from the results of earlier workers on other plants.

But when we made a routine check of the auxin transport in Coleus stems, we found to our discouragement that auxin movement was not strictly polar: significant amounts of auxin could move up the stem, as well as down (Jacobs, 1952).

Since Coleus had also been used by some of the researchers who reported strictly downward differentiation of xylem cells, the unexpected upward transport of auxin seemed to destroy the correlation on which our research was based. But just before dropping the problem, we thought we should check the direction of xylem differentiation. When we collected regenerating stems every day during the regenerating period, we found that xylem differentiation was not strictly downward either: shortly before the

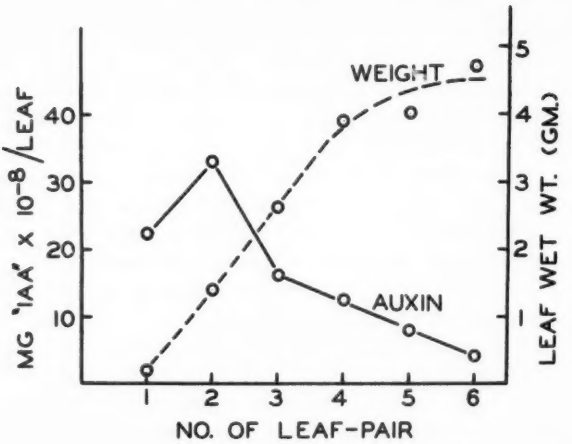

FIGURE 1. Amounts of auxin diffusing out of Coleus leaves of different positions and sizes, to show the relative amounts coming from different leaves.

downward differentiating strands reached the still intact portion of the xylem strand, short files of xylem cells would regenerate up to meet them. Thus, the correlation between auxin movement and xylem regeneration was even closer than the literature had originally suggested.

If leaves were cut off (removing sources of auxin), there was a decrease in amount of xylem regenerated. If synthetic auxin, in the form of indoleacetic acid, were added to plants with leaves removed, the amount of xylem increased again.

These qualitative results support the interpretation that auxin formed in the leaves is the normal limiting factor for the differentiation of xylem cells during the regeneration of a xylem strand. When leaves are present as sources of auxin both above and below the regenerating internode (as in the normal condition), most of the regeneration is downward, with a

smaller upward component, thus paralleling the ability of the stem to transport auxin (Jacobs, 1952).

Let us turn now to the quantitative relations.

QUANTITATIVE RESULTS

For this work, we used rigorously standardized material from a clonal stock of Coleus, thereby drastically reducing the variability. In addition, the samples were large in terms of most research on plant anatomy, and statistical tests of significance were routinely used.

Auxin-transport and xylem regeneration. When matched sections are cut from the standard internodes, and a concentration of synthetic indoleacetic acid slightly above the transport-capacity is added at either the top or bottom of the section, the amount of auxin collected at the other end is 65 units for "downward" (basipetal) transport, 20 units for "upward" (acropetal) transport. (A unit of auxin, as used here, represents 1×10^{-4} mg. IAA.) That is, when the same amount of auxin is available, more than three times as much can be transported basipetally as acropetally (Jacobs, 1954).

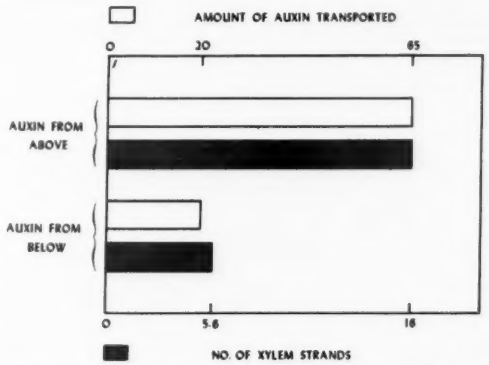

FIGURE 2. Relations between amount of auxin transported in isolated sections and the number of xylem strands regenerated in the plant when leaves above or below are excised.

Now let us see how the removal of the normally produced auxin supply affects the amount of xylem regenerated. This we can achieve by excising all the leaves either above or below the regenerating internode. If all the leaves above the wound are left on, an average of 16 strands of xylem are formed. (The number is not changed by removing the leaves below the wound.) If, on the contrary, the leaves above the wound are cut off while the leaves below the wound are left intact (thus leaving a source of acropetally moving auxin), then the number of xylem strands regenerated drops to an average of 5.6 strands. Figure 2 shows these results as they relate to the capacity of the stems for auxin transport in a basipetal vs. acropetal direction. It is obvious that there is a surprisingly exact parallel. The

amount of xylem regenerated is proportional to the transport-capacity of the internode for auxin (Jacobs, 1954). In other words, it looks as if auxin is the limiting factor for xylem differentiation, but how much of it actually gets to the wound site seems to be controlled by the amount of auxin that the stem can transport.

Rate of auxin-production. Since the level of production is usually presumed to control how much auxin is available for auxin-controlled processes, it is now of interest to compare the normal level of production by the leaves with the transport-capacity of the stems for auxin.

Theoretically, there are three possibilities:

1) The level of production could be more than the transport-capacity;
2) the level of production could more or less equal the transport-capacity;
3) the level of production could be substantially less than the transport-capacity.

The third possibility we can immediately discard, since we would not have obtained the observed correlation between transport-capacity and xylem

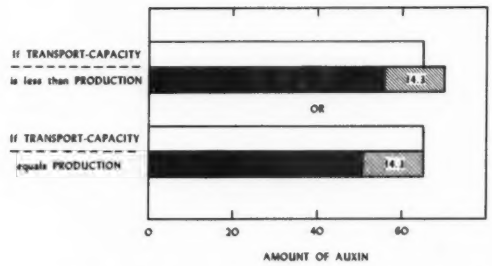

FIGURE 3. Graph illustrating the two possible effects which might be expected when part of the distal auxin supply is removed (by excising leaf 2 with its production of 14.3 units of "IAA" per hr.).

regeneration if the leaves were normally producing *less* auxin than the transport-capacity. So, production equals, or is larger than, the transport-capacity of the stem. Which is it? We can get evidence this way: if the leaves are producing auxin at a rate just matching the transport-capacity of the stem, then removing part of the auxin supply (as by cutting off only one leaf) should give a decrease in the amount of xylem which exactly and completely reflects the amount of auxin removed (as in the bottom half of fig. 3). But, on the other hand, if production is greater than transport-capacity, then removing one leaf will not give an exactly corresponding decrease in the amount of xylem regenerated, because part or all of that removal will be "used up" in just getting production down to the level of what the stem can transport (top half of fig. 3).

The amounts of auxin produced by the leaves just above the wounded internode are shown in figure 4. The leaf immediately above the wound (Leaf 2) produces an average of 14.3 units of "indoleacetic acid" per hour; the next leaf above that (Leaf 1) produces 8.0 units per hour. Now we need a quantitative measure of how much auxin gets into the plant when we add a known concentration of synthetic auxin in the form of indoleacetic acid. The method shown to the right of figure 4 was devised as a way of determining the physiologically significant amount of added indoleacetic acid that is transported from the site of application. Sections were cut from the leaf stalks of the standardized No. 2 leaves, indoleacetic acid was added to the leaf-blade (i.e., distal) end of those excised sections, and a block of agar was placed on the stem-end to collect auxin, as with the leaves to the left. As figure 4 shows, the addition of 2 p.p.m. of

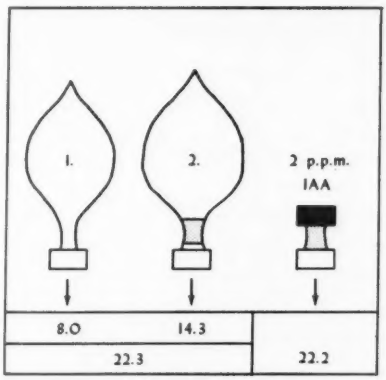

FIGURE 4. The average amounts of auxin obtained per hour from No. 1 and No. 2 Coleus leaves and from a petiole section from No. 2 leaf which was capped distally with 2 p.p.m. indoleacetic acid.

indoleacetic acid to the distal end gives 22.2 units per hour at the proximal end—an amount exactly the same as the combined production of leaves 1 and 2.

If we cut off all the distal leaves (i.e., leaves No. 1 and 2, plus the 4-5 pairs of still smaller leaves in the apical bud), then add through the leaf stalk of leaf No. 2 this 2 p.p.m. of synthetic indoleacetic acid, providing 22 units of physiologically significant auxin, a marked increase is seen in the number of xylem strands regenerated. The increase is 35 per cent of the number of strands regenerating in the control plants with all leaves on.

If production equals transport-capacity, excising leaves 1 and 2, with their 22 units of auxin, should give a *decrease* of 35 per cent in the number of xylem strands. Figure 5 shows that this is exactly the case.

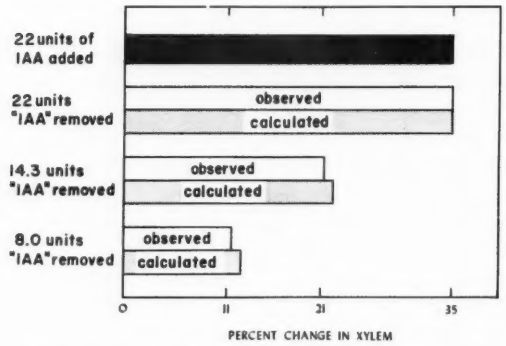

FIGURE 5. Graph showing effects on number of xylem strands regenerated per week of adding synthetic IAA, or of excising leaves 2 and/or leaves 1 (producing 14.3 and 8.0 units of "IAA" per hr., respectively). The data are given as per cent of strands regenerating in control plants with all leaves intact.

The bottom part of figure 5 also shows that the expected relations are found in other experiments where only leaf 2 is removed (with its 14.3 units of auxin) or where the 8.0 units from leaf 1 are removed (cf. Jacobs, 1956, for details).

These experiments, then, confirm the hypothesis that the leaves produce auxin at a level which normally just matches the transport-capacity of the stem below them. In other words, we cannot say that either auxin production or transport-capacity is *the* normal limiting factor for the differentiation of these xylem cells; normally they are both at the same level.

Auxin and non-regenerative xylem differentiation. When considering the evidence given above that auxin normally controls xylem differentiation during regeneration, one naturally wonders whether a similar relationship holds between auxin and xylem in the normal, non-regenerative development. As we have already seen (fig. 1), leaves of different sizes (and ages) produce different amounts of auxin. Leaves of the size and age which we have arbitrarily named "Leaf No. 2" produce auxin at the fastest rate.

If auxin is the limiting factor for xylem differentiation during *ordinary* development, then the number of xylem cells formed at the base of these different sized leaves should vary directly with the auxin production of the leaves.

Such counts were made recently by Paul Green. When the rate of formation of xylem cells formed at the base of the petioles was plotted against leaf size, the graph parallels the auxin curve shown in figure 1.

Thus, this first check supports the hypothesis that the differentiation of xylem during normal development is also limited by auxin from the leaves.

DISCUSSION AND SUMMARY

The most obvious conclusion from these experiments is that auxin is the normal limiting factor for the differentiation of xylem cells, the evidence being particularly extensive and quantitative with respect to xylem differentiation during regeneration of a severed vascular strand. The auxin controlling xylem differentiation is produced in the leaves and subsequently transported to the site of regeneration. The *entire* effect of the leaves in causing xylem regeneration is quantitatively explicable in terms of their production of auxin (when calculated as "indoleacetic acid"—cf. Jacobs, 1956, for further details).

The amount of auxin available for xylem regeneration is subject to control by both the amount produced in the leaves and the amount which the stem can transport. These two, transport-capacity and production, normally match, thus providing a "double check" on the auxin levels in the plant. (For a further discussion of the possible significance of the "double check" control, see Jacobs, 1956.)

A final point concerns the histochemical nature of auxin action. Auxin was first investigated, and is most widely known, as a hormone affecting growth. Most of the effects subsequently shown to be due to auxin application are also growth-promoting effects. This makes the clear relationship of auxin to xylem differentiation all the more puzzling, since apparently little or no growth of the parenchyma cells occurs prior to their transformation into xylem—at most a division or two precedes direct differentiation (Sinnott and Bloch, 1945). The most obvious change in these cells as they differentiate into xylem is the laying down of a thick secondary wall inside the thinner primary wall. This fact seems particularly significant when one remembers that auxin has an unusual position from the viewpoint of comparative biochemistry: unlike so many cell metabolites, it seems to have high activity only in plants—that is, among the organisms with cell walls. And the only group of plants where there is some question about the normal participation of auxin in growth is the fungi, plants with chitinous rather than cellulosic walls. These facts suggest that in its effects on both growth and xylem differentiation auxin is acting primarily on the cell wall.

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THE ORIGIN OF THE CULTIVATED CUCURBITA[1]

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INTRODUCTION

A symposium dedicated to Dr. Liberty Hyde Bailey is an appropriate occasion to discuss the Cucurbita. As a youth Dr. Bailey was fascinated by these, "marvels of the vegetable world," as he termed them. A sustained interest in this group of plants is suggested by his record of published research relating to the Cucurbita, commencing in 1890 and terminating in his monograph, "Species of Cucurbita," which appeared in 1943. In the intervening years, about one-half dozen papers were devoted to squashes, pumpkins and gourds. Some of Dr. Bailey's observations on these lusty, rapidly growing vines, with an apparently modest root system make interesting reading today. For example, "One spring I planted a single seed of a gourd in my greenhouse. It covered a long bench, ran out the ventilators at the top of the house, down the roof on the ground, full 100 feet by the time frost overtook it." Commenting on his experience of over 40 years with pumpkins, squashes and gourds he stated: "More than 1000 experiments were made, including several hundred hand crosses, and probably as many as 2000 nondescript forms of pumpkins and squashes were produced. The extent of the effort terminated itself, for the results were too voluminous to publish in full."

Except for that of corn, the archeological record of the cultivated Cucurbita is more abundantly documented than that of any other crop plant. Peduncles, rinds or shells, and seeds make excellent archeological subjects. The earliest archeological record from South America is that of *C. moschata* and *C. ficifolia* from Huaca Prieta, a site along the arid coastal region of Peru (Whitaker and Bird, 1949). This site has been dated by conventional methods as about 3000 B.C. In North America, the earliest record of Cucurbita is from the Ocampo Caves near Tamaulipas, Mexico (Whitaker, et al, in press). At the lower levels of this site, dated as about 3000 B.C., stems, rinds and seed of *C. pepo* have been recovered.

The American origin of all species of the cultivated Cucurbita is now established beyond reasonable doubt (Whitaker, 1947). Some data suggest that Central America and southern Mexico may have been the center of

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origin and domestication of this group (Vavilov, 1931). At any rate this would seem to be a likely area to search for evidence of this nature.

The cultivated Cucurbita have much to offer the student interested in the origins of crop plants. In spite of their general similarity, five[2] species can be distinguished on the basis of their morphological characters. No single character can be used to separate all the species, and there are many overlapping characters. However, when good herbarium specimens, along with seeds and a photograph of the fruit, are present for examination, the specimens can be assigned to a particular species with a great deal of precision.

All species of Cucurbita examined have 20 pairs of chromosomes. The chromosomes are small and are not easily manipulated by the usual methods. For these reasons it has been necessary to develop special techniques adapted to this material (McGoldrick, Bohn and Whitaker, 1954).

The compatibility relationships of the several species have been investigated to the point where the overall picture is becoming increasingly clear. With one exception, interspecific matings produce sterile F_1 progenies and for the most part backcrosses to either parent are either sterile or sparingly fertile (Whitaker and Bohn, 1950, and unpublished data). The data from interspecific crosses suggest that each species has built up a system of sterility barriers capable of preventing gene flow between them, hence permitting the several species to maintain their identity even though some of them have been grown side by side for centuries. Nothing is known about the physiological basis of the sterility barriers, although there are a few experiments that indicate that in some species foreign pollen does not grow rapidly enough on the stigma and in the style of the recipient to effect fertilization. The diminutive embryos obtained in most interspecific crosses suggest that faulty endosperm development may be responsible for the small, starved embryos.

EXPERIMENTAL RESULTS

Description and cross-compatibility relationships of C. lundelliana. After several non-productive attempts to hybridize the cultivated species with a number of annual and perennial wild species, seed from four collections of the PETEN GOURD, *Cucurbita lundelliana* Bailey, was received in 1952. This species is reported to be widespread in Central America, notably in southeastern Mexico (Campeche), Guatemala and British Honduras (Bailey, 1943). *C. lundelliana* is an attractive annual with relatively thick, fleshy roots. The stems are slender and long-running and

[2]The five cultivated species with the common names of their forms are:

annual
- *C. pepo* L.—summer squash, a few winter squash, pumpkin, ornamental gourd
- *C. moschata* Duch.—winter squash, pumpkin
- *C. mixta* Pang.—winter squash, pumpkin, cushaw
- *C. maxima* Duch.—winter squash, pumpkin

perennial
- *C. ficifolia* Bouché—fig-leaf gourd, Malabar gourd

root at each node. Typical leaves are deeply 5-lobed, dull green, thin and softly hirsute, with entire margins; the petiole is slender with soft hairs. The staminate flowers are large and handsome on long, slender pedicels; the pistillate flowers are similar in appearance, but with shorter, stouter pedicels. The fruit is a small (7.5 × 7.0 cm), green, round, hard-shelled gourd, slightly tapered at the stem end, with bitter, stringy, greenish-white flesh. The peduncle is sharply angled and furrowed, but not flared at the fruit attachment. The seeds are small (10 × 5 mm), ash-grey, with a broad heavily sculptured margin, tapering to an acute base.

The significant geographical distribution of *Cucurbita lundelliana* suggested at once that its relationship to the cultivated species might be worth investigation. Accordingly, pistillate flowers of *C. lundelliana* were pollinated with staminate flowers from each of the five cultivated species. Surprisingly the pistillate flowers of *C. lundelliana* set fruits readily regardless of the pollen source. The F_1 and the progenies of the backcross to each parent from three of these matings have been grown and studied (*C. lundelliana* × *C. maxima*, *C. lundelliana* × *C. moschata*, and *C. lundelliana* × *C. ficifolia*). The other two (*C. lundelliana* × *C. mixta* and *C. lundelliana* × *C. pepo*) have produced fruits with abundant seeds, but the embryos are small. Special techniques, probably embryo culture, will be required to produce F_1 plants from this material.

At this time it is not proposed to examine the behavior of each cross and the resulting progeny in detail, but rather to discuss the significance of the observations as they are related to the origin of the cultivated group.

Pollen fertility of the F_1 hybrids. There is a wide variation in pollen abortion of the F_1 hybrids from the various crosses as indicated by the percentage of stainable pollen in aceto-carmine smears. This variability depends upon the species used as a pollen parent with *Cucurbita lundelliana*. When *C. moschata* is used, 42 per cent of the pollen from the F_1 plants is normal; 17 per cent in the cross with *C. maxima*; and only 15 per cent when *C. ficifolia* is used as one parent. In general, more plump seed per fruit was obtained from F_1 plants with the highest percentage of stainable pollen, as would be expected.

Cytology of the F_1 hybrids. A thorough study of meiosis in the three species crosses that produced F_1 hybrids has not been completed. However, enough has been done to indicate that meiosis in each is uncommonly regular. For the most part there are 19 or 20 bivalents; in a few cells an occasional quadrivalent and as many as 4 unpaired chromosomes in some cells may occur. In the F_1 from the cross *C. lundelliana* × *C. maxima* bridge-fragment configurations were fairly common.

DISCUSSION AND CONCLUSIONS

The pattern formed by these compatibility relationships is sufficiently unusual to deserve comment. Here we have five cultivated species, rather similar in their gross morphological characters and for the most part cross-

incompatible among themselves, but cross-compatible with a non-cultivated species. Furthermore, the F_1 hybrids from three of these species crosses are self-fertile and cross-fertile with each parent.

The results of the crosses are indicative of the genetic affinities between the cultivated species. On the basis of their mating behavior with *C. lundelliana* they separate sharply into two groups. The first group consists of three species (*C. moschata*, *C. maxima* and *C. ficifolia*). In this group, matings produce seeds with normal embryos and the F_1 plants are self-fertile and cross-fertile with either parent. In the second group of two species (*C. mixta* and *C. pepo*) the F_1 seeds produce small embryos that do not emerge from the seed coats under the usual conditions for good germination.

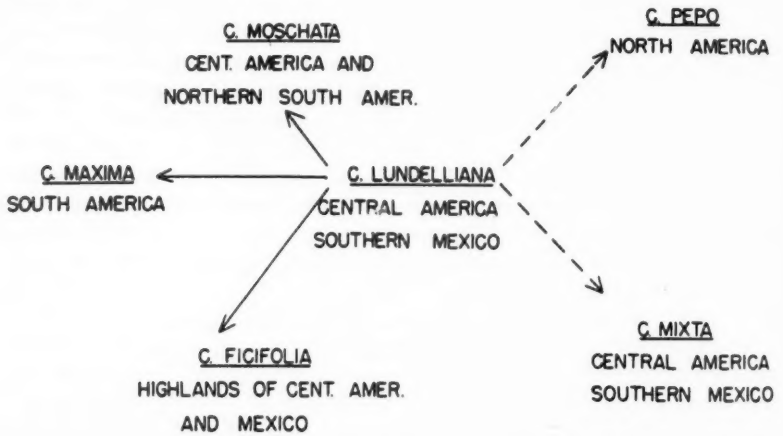

FIGURE 1. The geographical interrelationships of the five cultivated species of *Cucurbita* with *C. lundelliana*. (See further explanation in the text.) The solid lines indicate that F_1 hybrids with *C. lundelliana* produce seeds with normal embryos; the broken lines indicate that seeds with small sub-normal embryos are produced.

This separation into two groups suggested by the results of the matings with *Cucurbita lundelliana* is strengthened by the breeding behavior of the five cultivated species among themselves. The hybrids obtained from crosses between *C. maxima* and *C. pepo* and *C. maxima* and *C. mixta* resemble one another in contrast to hybrids obtained by mating *C. maxima* with *C. moschata*, where the fruit from the F_1 hybrids is altogether different in appearance. Furthermore *C. maxima* crosses readily with *C. moschata*, producing partially fertile F_1 hybrids and self-fertile, stable amphidiploids. It crosses with *C. mixta* and *C. pepo* only with difficulty. The F_1 hybrids from these matings are nearly sterile, and the amphidiploids are unstable and sparingly fertile. *C. ficifolia* occupies a central position as it crosses with *C. maxima* in both directions and with *C. pepo* only

when *C. ficifolia* is used as the female parent. However, the hybrids from these crosses are completely sterile (Weiling, 1955).

The geographic interrelationships of the five cultivated species with *C. lundelliana* are diagrammed in figure 1. At the extremes are *C. maxima* in South America and *C. pepo* in North America. In the central portion of the hemisphere are *C. moschata* in Central America and northern South America and *C. mixta* in Central America and southern Mexico. These are all lowland species. In the highlands of Central America and Mexico, *C. ficifolia* is found.

We assume that these studies have some practical value, possibly for the plant breeder. For instance, *C. lundelliana* might be utilized as a bridge to transfer certain desirable characters of *C. pepo* to *C. maxima*: for example, squash bug resistance. This transfer could probably be done at the diploid level, and in all likelihood satisfactory fertility could be retained. Moreover, tolerance to powdery mildew and the thrifty, vigorous vine habit of *C. lundelliana* might be easily transferred to the cultivated species by appropriate breeding procedures.

The cross-compatibility of *C. lundelliana* with each of the cultivated species suggests that they were derived from a common gene pool or at the very least that they must have an identical complement of several genes. The sterility barriers that are effective in maintaining the identity of the cultivated forms are negated in matings with *C. lundelliana*. Evidently this species is the common denominator of the entire group. The data from these experiments lead one to suspect that the sterility barriers which operate effectively in the cultivated species are confined to this group and are probably not present in the non-cultivated members of the genus.

Does this mean that *Cucurbita lundelliana* may have been the ancestor of the cultivated group? Our studies have not progressed to the point where we can give an informed answer to this question. However, there are two pieces of evidence that support an affirmative answer: (1) the distribution of *C. lundelliana* in Central America and southern Mexico coincides with the hypothesis that the center of origin of the cultivated Cucurbita is to be sought in this general region; (2) the hybridization experiments indicate some measure of compatibility between *C. lundelliana* and each of the five species of cultivated Cucurbita.

SUMMARY

The five cultivated species of Cucurbita (*C. pepo*, *C. mixta*, *C. moschata*, *C. ficifolia* and *C. maxima*) are very much alike in their morphological characteristics, but they are for the most part cross-incompatible. They have apparently developed a sequence of sterility barriers that effectively prevent gene flow between them. In a series of interspecific matings, the five cultivated species are shown to be cross-compatible with a wild species, *C. lundelliana*, from southern Mexico and neighboring Cen-

tral America. Matings between *C. lundelliana* and *C. moschata*, *C. maxima* and *C. ficifolia* produced self-fertile F_1 plants that were cross-fertile with either parent. The other two matings (*C. lundelliana* × *C. mixta* and *C. lundelliana* × *C. pepo*) produced fruit, but the seeds had small embryos. The data from interspecific hybridization and those of geographical distribution suggest that *C. lundelliana* may have had an important role in the origin of the cultivated group.

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CRYPTIC COLORATION IN THE ANTHOPHILOUS MOTH *RHODODIPSA MASONI**

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Most Heliothidine moths are characterized by contrasting coloration, by association with one or few species of plants, and by cryptic resemblance to the flowers of these plants (Grote 1882; Bird 1923; Kwiat 1908; Wyatt 1938, 1941). Furthermore, the larvae of each species usually feed on the flowers and seeds of the same plant species on which the adults are characteristically found. Cockerell (1910) was the first to record the particularly striking resemblance of the adult *Rhododitbsa masoni* J. B. Smith (Lepidoptera, Noctuidae, Heliothidini) to the inflorescence of the Composite, *Gaillardia aristata* Pursh, on which it always was found. He mentioned that the moth rests on the flower in such a way "that although the insect is brightly colored, it becomes practically invisible." The present paper is a quantitative study of this color relationship.

The authors wish to express their appreciation to Dr. C. L. Remington for his helpful criticism and discussion in the writing of this paper, and to Drs. E. B. Ford and P. M. Sheppard for their enthusiastic suggestions while in Colorado. We want to thank Dr. J. W. Marr of the Institute of Arctic and Alpine Ecology, University of Colorado, for arranging our research facilities at Science Lodge. We also appreciate the kind help of Messrs. J. G. Coutsis, D. Eff, R. R. McElvare, and A. K. Wyatt. This work was carried out as an adjunct to research in Colorado aided by a grant from the Sigma Xi-RESA Research Fund.

All recorded observations were made between 15 June and 10 July 1955, in the foothill and montane zones of the Rocky Mountains in Boulder County, Colorado. The moths were first observed by us on the flowers of *G. aristata* on 20 June 1954, at an altitude of approximately 8500 feet, along a road built on top of the old Denver-Rio Grande Railroad bed just west of Sugar Loaf Mountain. In 1955, observations were made along this road, the road from Boulder Canyon to Sugar Loaf Mountain, the road from Colorado Highway 160 to Gold Hill, and in Buckingham Park at the bottom of Left Hand Canyon. The approximate altitudinal range of all moths found was 5900 feet in Buckingham Park to 8700 feet along the road to Gold Hill. Altitudes were taken from the 1904 U.S.G.S. Boulder Quadrangle Map. The moths were found only where blooming *G. aristata* also occurred.

*This paper is dedicated to Professor Alexander Petrunkevitch on the occasion of his 80th birthday.

The moth is conspicuously colored. Both head and thorax are Cadmium Yellow which contrasts strikingly with the predominantly Ox-blood Red forewings. The color of the hindwings (Dark Vinaceous-Brown) is unimportant here since they are covered by the forewings while the moth is feeding and when it is at rest. (These colors were determined by comparison to Ridgway's (1912) color tables.) The inflorescence of *Gaillardia aristata* is colored in the following general manner: the ray florets are yellow; red disk florets occur in a concentric band just inside the ray florets, and yellow disk florets compose the center of the flower. Thus

FIGURE 1. *Rhododipsa masoni* adult at rest on inflorescence of *Gaillardia aristata* in the typical "head-out, inconspicuous" position. Approx. 1.5 natural size. For the moth, rfw = red forewing; yt = yellow thorax; for the Composite flower, yrf = yellow ray floret; rdf = red disk florets; ydf = yellow disk florets. (Photograph by the authors).

from the periphery to the center, the flower is yellow, then red, then yellow. The size, color, and color proportions of any one flower vary throughout development, and even at any one stage of development there is variation from flower to flower. The yellow and red colors of the moth and the flower are very similar, but not identical. The accompanying photograph (figure 1) shows a moth on a flower which is at an early stage of development.

It was noted that a moth could be either conspicuous or inconspicuous on a *G. aristata* flower depending on how it was oriented. It was inconspicuous if its yellow anterior part was over either the yellow ray florets

("head-out," see figure 2, position 1), or over the yellow central part of the disk ("head-in," see figure 2, position 2). In these two positions the colors of the moth and flower blended, and the moth was much less conspicuous than when it was oriented "sideways" (see figure 2, position 3). In the "sideways" position, the yellow anterior part contrasted with the underlying red disk florets, thereby making the moth conspicuous. How-

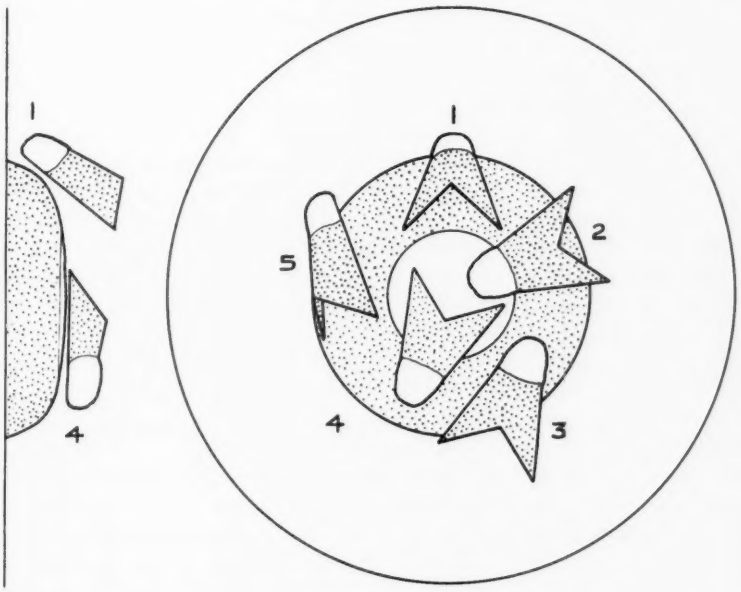

FIGURE 2. On right: Top view. Diagrammatic representation of moths on an inflorescence to indicate the relationship of various positions to conspicuousness. Stippled area = red, open area = yellow. 1: head-out, inconspicuous; 2: head-in, inconspicuous; 3: sideways, conspicuous; 4: head-out, conspicuous; 5: sideways, inconspicuous. On left: Side view of same. For clarity, only moths in positions 1 and 4 are shown.

ever, in a few cases the conspicuous or inconspicuous condition of the moth was not in agreement with the orientations just described (see figure 2, positions 4 and 5). Therefore each moth was recorded both according to its class of orientation, *i.e.* "head-in," "head-out," or "sideways," and according to whether the colors of the moth contrasted or harmonized with those of the Gaillardia flower, *i.e.* "conspicuous" or "inconspicuous" (see table). Moths not on the upper surface of *G. aristata* flowers were recorded according to the substrate on which they were found. Although considerable time was spent observing other plant species in the same areas, no moths were found on any except those listed in the table. It is assumed that each observation represented a different moth. This assump-

TABLE

DISTRIBUTION AND APPEARANCE OF 26 *RHODODIPSA MASONI* ADULTS ON VARIOUS SUBSTRATES DURING DAYTIME.

On upper surface of *Gaillardia aristata* flower						On other substrates			
Head-out		Head-in		Sideways		Underside *G. aristata* flower	Green leaf *G. aristata*	Green leaf *Alnus tenuifolia*	Bud *Cirsium undulatum*
Incon.	Con.	Incon.	Con.	Incon.	Con.				
14	2	2	0	1	2	2	1	1	1

Total Inconspicuous 17
Total Conspicuous 4

tion seems justified, because the records were taken over a wide area at different times and often were made simultaneously by two observers.

On the basis of random scattering, one would expect to find an equal number of moths on the upper surface of *G. aristata* flowers and on each of the other four substrates included in the table. However, 23 out of 28 moths were observed on top of the flowers (an additional 2 moths not included in the table flew off the upper surface of *G. aristata* flowers, raising the total observations from 26 to 28). The probability that this distribution occurred by chance alone is less than .001. Thus it is clear that *R. masoni* is associated with the upper surface of *G. aristata* flowers. A comparison of the moths which were inconspicuous (17) to those which were conspicuous (4) indicates that the number inconspicuous deviates significantly from the number expected on the basis of a .5–.5 distribution ($P < .005$). This supports the contention that the moth is in a cryptic position when on the upper surface of *G. aristata* flowers. If the 6 positions of the moths on the upper surface of the flowers are compared, it is seen that the distribution in these columns deviates significantly from the hypothesis that a moth has an equal chance of acquiring any 1 of the 6 positions when it is on the upper surface ($P < .001$). However, if the first column ("head-out, inconspicuous") is excluded and all the remaining columns compared, it is seen that the moths are randomly scattered throughout the 9 columns. Thus, a non-random distribution of the moths results from the predominant number of moths in the "head-out, inconspicuous" position on the flower.

DISCUSSION

Seen as a museum specimen, this small moth with its bright contrasting colors might seem a protected species advertising its noxious qualities through its bright warning coloration. However, Cockerell's note (1910) and the data now presented clearly indicate that the coloration of *R. masoni* is, in fact, cryptic due to its intimate relationship with the brightly colored flowers of a specific Composite plant. The details of this cryptic coloration are exemplified by a comparison of the visual textures of the flower

and the moth. Each flower is composed of numerous units which together present a mosaic appearance; a similar effect is produced by the mottled forewing pattern of the moth. This fact, plus the red-yellow color division of the moth, correlated with its typical orientation produces a remarkable resemblance to the various parts of the flower (see figure 1).

It was pointed out above that a moth could be cryptic in either a "head-out" or a "head-in" position, but the data indicated a preponderance of the moths in the "head-out" position. This seems to be correlated with the mode of feeding of the moths and with the blooming sequence of the inflorescence. The florets of the disk mature in sequence towards the center. The moths were observed feeding radially on the mature peripheral florets of the disk, while at the same time the central florets were immature and offered no source of nectar. At the end of feeding, each moth usually maintained the "head-out" orientation and settled down with its yellow anterior region adjacent to or over the yellow ray florets and its red wings over the red disk florets. Although the possibility exists for moths to feed in a "head-in" orientation, this is unlikely because the ray florets were observed to be mechanically unsuited as a feeding platform. Thus the typical centrifugal orientation of the moth appears to be related to its mode of feeding which in turn seems to be determined by the centripetal blooming sequence of the individual florets of the Composite. The data presented suggest that the habit of feeding head-out on the disk of the Gaillardia flower preceded the evolution of the bicoloration of the moth. It seems likely that natural selection established the bicoloration of the moth for better cryptic resemblance because its normal feeding orientation on the Gaillardia flower placed it on a bicolored background.

SUMMARY

1. Data are presented which show the intimate relationship of a bicolored moth with a similarly bicolored Composite flower.

2. The typical orientation of the moth on the flower is such that its colors blend with those of the flower; the moth therefore possesses cryptic coloration on the flower.

3. This color orientation of the moth arises from the way in which the moth feeds on the Composite flower and this feeding position in turn is determined by the blooming sequence of the individual florets.

4. It seems likely that selection favored evolution of the bicoloration of the moth because its normal feeding orientation on the Gaillardia flower placed it on a bicolored background.

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A NUTRITION-INHIBITION HYPOTHESIS OF PATHOGENICITY

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The categories of parasitism may be determined by the type of response exhibited by the host to an entry or to the presence of the parasite. Pathogenicity may be distinguished from saprophytism and symbiosis in that the host is adversely affected by the parasite. It is possible for a given relationship to shift from one category to another under certain conditions. The successful invasion and subsequent extensive multiplication or metabolism in a host with its characteristic attendant reactions are the basis for considering a parasite to be pathogenic and simultaneously, the host to be susceptible. On the other hand, if the parasite fails either to proliferate or to metabolize extensively after invasion, this may reflect either the avirulence of the parasite or the resistance of the host or both. Consequently, pathogenicity must always be defined as an expression of a two component system: the host-parasite relationship. Such a definition which also applies to the other categories of parasitism is basically ecological, emphasizing the host as an environment for the parasite.

Pathogenicity as it concerns the parasite assumes that the parasite is capable of utilizing the host environment as a growth medium and that the parasite can overcome the total defense mechanisms of the host. It should be noted that two problems are involved: the ability of the parasite to proliferate or to metabolize extensively and the mechanism(s) whereby the parasite damages either the host *in toto* or certain cells or tissues of the host. The host may be resistant either if it does not provide a suitable growth medium for the parasite or if the host presents defense mechanisms which prevent, inhibit, or restrict the potential proliferation or metabolism of the parasite. These considerations have led to the formulation of a nutrition-inhibition hypothesis of pathogenicity. Lewis (1953) has presented a comparable hypothesis from a different point of view in his "balance hypothesis of parasitism."

The nutrition-inhibition hypothesis of pathogenicity involves the two environments in the host which directly affect the fate of an invading parasite: a nutritional environment and an inhibitory environment. If the nutritional environment is inadequate for the parasite, the latter can neither proliferate

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nor metabolize extensively and will not be virulent; if the nutritional environment is adequate for the parasite, the latter will be able to proliferate or to metabolize extensively but may or may not be virulent. An effective inhibitory environment may or may not also be present. In the absence of an effective inhibitory environment, a parasite in an adequate nutritional environment generally will be virulent; in the presence of an effective inhibitory environment, the parasite will not be virulent even though the nutritional environment is adequate. Of the four possible combinations of host environments, only the adequate nutrition-ineffective inhibition environment results in virulence; the other environments result in avirulence. The antigenic, morphological, and enzymatic characteristics of the parasite add complications to be discussed later.

NUTRITIONAL REQUIREMENTS OF THE PARASITE

The role of nutrition in the host-parasite (pathogen) relationship will be referred to the nutritional requirements of the parasite as they may or may not be satisfied by the host environment. The parasite must get all of its nutritional requirements from the host either at the site of inoculation or of localization. Many pathogens have nutritional requirements beyond the basic need for certain elements and an energy source, indicating that they no longer synthesize certain materials such as amino acids, vitamins, growth factors, *etc*. The relationship between the host as a growth medium and the specific nutritional requirements of the parasite, including animal and plant pathogens, has been subjected to direct study only comparatively recently (Bacon, Burrows, and Yates, 1950, 1951; Garber, Hackett, and Franklin, 1952; Garber, 1954; Keitt and Boone, 1954).

Considerable effort has been expended in clarifying the role of such inhibitory environments as the humoral and cellular defense mechanisms of animal hosts. Although varieties and species of plants resistant to certain parasites have long been known, the mechanisms conferring resistance on these hosts are not yet adequately understood. The defense mechanisms of the host constitute the potentially inhibitory environment which the parasite must overcome if it is to be pathogenic. It should be emphasized that a specific defense mechanism effective for a specific parasite may be ineffective for another parasite. Also, defense mechanisms need not be absolute in their effectiveness.

Any hypothesis concerning pathogenicity must also consider within its framework non-pathogenic as well as pathogenic species or strains. Although the nutrition-inhibition hypothesis provides three distinct situations resulting in avirulence, it may be technically difficult to assign a specific example of avirulence to a specific situation. Furthermore, the parasite may alter its immediate environment within the host. For example, the animal host may produce antibodies or antitoxins as the parasite is proliferating or metabolizing; the plant host may produce structures limiting the spread of the inoculum. The nutritional-inhibitory environments in the

host may be influenced by the host's environment. Consequently, examples of the possible situations indicated by the nutrition-inhibition hypothesis may be unique in their simplicity. These examples, however, may serve as models for future investigations.

VIRULENCE

A parasite confronted with an adequate nutritional environment and an ineffective inhibitory environment generally will be virulent. If the parasite proliferates or metabolizes extensively in a host, the host must be providing all the nutrilites required by the parasite; a susceptible host *ipso facto* presents an ineffective inhibitory environment. Experimental evidence indicating that the host is indeed providing specific nutrilites has come from studies using biochemical mutants of animal and plant pathogens (*Salmonella typhosa,* Bacon *et al.,* 1950, 1951; *Klebsiella pneumoniae,* Garber *et al.,* 1952; *Erwinia aroideae*, Garber, 1954; *Venturia inaequalis*, Keitt and Boone, 1954; *Agrobacterium tumefaciens*, Garber *et al.*, 1955). These experiments employed biochemical mutants with specific requirements for an

TABLE 1

RELATION BETWEEN VIRULENCE AND NUTRITIONAL REQUIREMENTS OF BIOCHEMICAL MUTANTS OF *KLEBSIELLA PNEUMONIAE* INOCULATED INTO MICE EITHER INTRAPERITONEALLY OR INTRANASALLY (GARBER *ET AL.*, 1952).

Mutant	Need	Route of Inoculation			
		Intraperitoneal		Intranasal	
		Dosage	Dead/Total	Dosage	Dead/Total
"wild"	none	1×10^4	9/10	3×10^5	9/10
34	threonine	1×10^4	10/10	3×10^5	10/10
2	tyrosine	1×10^4	10/10	3×10^5	10/10
40	methionine	1×10^4	9/10	3×10^5	10/10
27	leucine	1×10^4	9/10	3×10^5	8/10
17	histidine	1×10^4	10/10	3×10^5	10/10
22	uracil	3×10^3	4/5	7×10^4	2/5
2711	quanine	3×10^4	0/10	8×10^5	0/10
58	adenine	1×10^5	0/10	3×10^6	0/10
58-01	none	1×10^4	9/10	3×10^5	9/10
58-02	none	1×10^4	6/10	3×10^5	9/10

amino acid, vitamin, growth factor, purine, or pyrimidine. For example, the virulence of biochemical mutants of *K. pneumoniae* was determined by inoculating mice intraperitoneally or intranasally. Mutants requiring threonine, tyrosine, methionine, leucine, histidine, or uracil (pyrimidine) retained their virulence (Table 1). Cultures obtained from the peritoneal fluid of animals succumbing after an intraperitoneal inoculation and from animals succumbing after an intranasal inoculation were tested to determine whether the specific nutritional requirement was still present. In all cases, the recovered cultures were still mutant, indicating that reversion to prototrophy (the parental type) had not occurred in the host. Finally, peritoneal

fluid from the mouse was tested for its ability to satisfy the nutritional requirements of these virulent, biochemical mutants and in each case, the nutritional requirement was satisfied. Consequently, a direct relationship between the nutritional requirements of these virulent, biochemical mutants and the availability of the specific, required nutrilite in the host either at the site of inoculation or of localization was established.

It has been possible to demonstrate an apparent increase in virulence by supplying either "preformed" materials or a greater amount of the substrate presumably required by the parasite in the host. Bacon *et al.* (1951) injected purine into the peritoneal cavity of the mouse along with the parental strain of *S. typhosa* which does not require exogenous purine and observed a significant increase in virulence. Berry and Mitchell (1953) introduced Krebs cycle poisons along with *Salmonella typhimurium* into the peritoneal cavity of the mouse and increased the virulence of the pathogen, presumably by increasing the concentration of the energy source available to the pathogen.

If the parasite cannot proliferate or metabolize extensively in the host, it cannot be virulent. Avirulence may result either from an inadequate sup-

TABLE 2

THE EFFECT OF INTRAPERITONEAL INOCULATIONS OF THE COMPLEX PURINE-LESS MUTANT, 27139, OF *KLEBSIELLA PNEUMONIAE* AND EITHER HYPOXANTHINE OR A PURINE POOL* (GARBER *ET AL.*, 1952).

Expt.	Treatment	Total Dosage	Dead/Total
I	27139	1.3×10^5	0/10
	hypoxanthine	50 mg.	0/10
	27139 plus	1.3×10^5	
	hypoxanthine	50 mg.	3/10
II	27139	8×10^6	0/10
	purine pool*	24 mg.	0/10
	27139 plus	8×10^6	
	purine pool*	24 mg.	10/10

*15 mg. hypoxanthine, 3 mg. xanthine, 3 mg. adenine, 3 mg. guanine.

ply of required nutrilites or the presence of an effective inhibitory environment. In some instances, it has been possible to distinguish between these alternatives. Avirulence may also result from both situations occurring together.

The greatly diminished virulence or avirulence of purine-requiring mutants of *S. typhosa* (Bacon *et al.*, 1951), *K. pneumoniae* (Garber *et al.*, 1952), and *Pasteurella pestis* (Burrows, 1955) reflects an inadequate nutritional environment in the host, the mouse. Burrows (unpub.) noted that a purine-requiring mutant of *P. pestis* is likewise avirulent for the guinea pig and the rat. The purine-requiring mutants of *K. pneumoniae* (2711, 58) were avirulent for the mouse when inoculated either intraperitoneally or intranasally (Table 1). Peritoneal fluid from the mouse did not satisfy the nutritional requirements of these biochemical mutants. Prototrophic reversions

(58–01, 58–02) from a purine-requiring mutant were virulent when inoculated either intraperitoneally or intranasally (Table 1). Finally, introducing purine and a purine-requiring mutant into the peritoneal cavity of the mouse resulted in a display of virulence (Table 2). Cultures recovered from the peritoneal cavity and lungs of dead mice were mutant, indicating that reversions to prototrophy were not responsible for the death of the mice. Moribund mice were sacrificed and only mutant cultures were recovered.

AVIRULENCE AND NUTRITION

Experiments with plant pathogens have provided confirmatory evidence that an inadequate nutritional environment may result in avirulence (Garber,

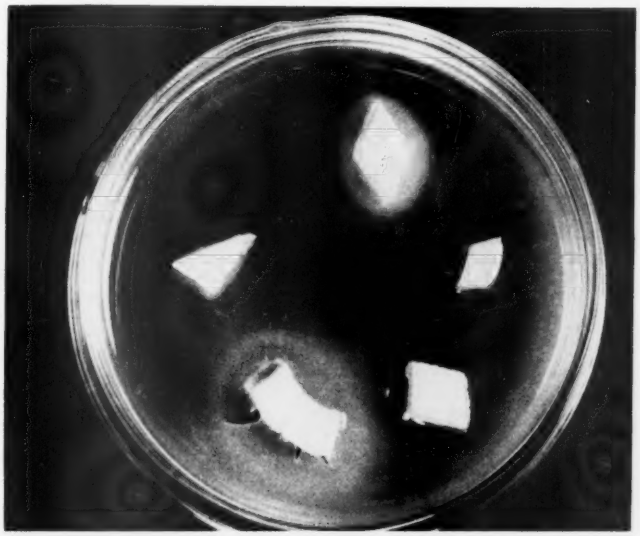

FIGURE 1. The response of an arginine-requiring mutant of *Erwinia aroideae* to blocks of storage organ tissue of the potato (susceptible), carrot (resistant), sweet potato (resistant), parsnip (susceptible), and radish (resistant). The potato tissue block is at 12 o'clock, the other tissue blocks occurring clockwise.

1954; Keitt and Boone, 1954). For example, an arginine-requiring mutant of *E. aroideae*, a bacterial plant pathogen, was avirulent for the radish and the turnip when the cut surface of the fleshy storage organ was inoculated (Garber, 1954). If a block of tissue from the storage organ was added to a culture of this mutant in the minimal agar, no bacterial growth was observed around the tissue block (Fig. 1). However, if a homogenate of the entire storage organ was added as a drop to a culture of this mutant in the minimal agar, growth was observed around the drop. These observations indicated that the required nutrilite was present *in* the tissue in sufficient concen-

tration to support the growth of the mutant but the concentration of the required nutrilite either at the site of inoculation or at the surfaces of the tissue block was inadequate to support the growth of the mutant. On the other hand, the arginine-requiring mutant was virulent for the potato. Growth was observed around the block of potato tissue added to a culture of the mutant in minimal agar (Fig. 1).

Avirulent strains or species of pathogens may proliferate or metabolize for some time after invasion and then stop either proliferating or metabolizing. Although an effective inhibitory mechanism may be assumed to be operating in the host, such observations may also reflect an inadequate nutritional environment. For example, an arginine-requiring mutant of *E. aroideae* was avirulent for the radish using a standardized procedure prior to inoculation (Garber, 1954). According to this procedure, mutant cells previously grown in nutrient broth supplemented with yeast extract were washed twice in 0.85 per cent saline prior to inoculation, a process lasting 2 hours. The following procedure was then adopted: the cells were washed once and then suspended in 0.85 per cent saline, a process lasting 30

TABLE 3

SYNERGISTIC EFFECT OF PURINE-LESS AND PARA-AMINOBENZOIC ACID-LESS (PABA) MUTANTS OF *SALMONELLA TYPHOSA* INOCULATED INTRAPERITONEALLY INTO THE MOUSE (BACON *ET AL.*, 1951).

Total dose of organisms	Deaths out of 20 mice inoculated with		
	Purine-less mutant	PABA-less mutant	Purine-less and PABA-less mutants
$\times 10^{-6}$			
100	0	0	2
200	0	1	17
400	2	1	18
800	9	10	20

minutes, and immediately incubated at 27° C. Arginine could not be detected as carry-over from the broth in the saline suspensions of cells. Inocula were then extracted every 15 mins. for a virulence test on slices of radish. The inoculum taken just prior to incubation served as a control. The control cells and the cells incubated for 60 mins. were virulent; the cells taken at 75 mins. were weakly virulent; and the cells extracted at 90 to 120 mins. were avirulent (Garber, unpub.). These results suggest that an initial, endogenous stockpile of arginine or materials yielding arginine was diminishing until it was inadequate to support growth in the absence of an exogenous supply of the required nutrilite. It had already been established that the site of inoculation lacked an adequate supply of arginine to support a proliferating population of arginine-requiring cells of *E. aroideae* (Garber, 1954).

Bacon *et al.* (1951) have provided an interesting example of the virulence of a mixed culture of two biochemical mutants of *S. typhosa*, each mutant itself being relatively low in virulence for the mouse (Table 3). One mutant required purine; the other mutant required PABA. If suboptimal amounts of the required nutrilites were supplied to a mixed culture of these mutants *in vitro*, growth was normal and a heavy suspension of cells resulted. This observation indicated a syntrophic effect, *i.e.*, the purine-requiring mutant excretes during its metabolism a material satisfying the nutritional requirement of the mutant requiring PABA and the latter mutant excretes in turn a material satisfying the nutritional requirement of the mutant requiring purine (Davis, 1950). If the host provides suboptimal concentrations of either purine or PABA, the nutritional requirements of these biochemical mutants

TABLE 4

EFFECT OF DIETARY PARA-AMINOBENZOIC ACID (PABA) ON THE VIRULENCE OF A PABA-LESS MUTANT OF *SALMONELLA TYPHOSA* INOCULATED INTRAPERITONEALLY INTO MICE (BACON *ET AL.*, 1951).

Culture	PABA given on days*					Challenge	Deaths
	5	4	3	2	1	Dose $\times 10^{-6}$	
PABA-less mutant	−	−	−	−	−	400	3
	−	−	−	−	−	100	0
	−	−	−	−	+	100	16
	−	−	+	+	+	100	18
	+	+	+	+	+	100	20
parental strain	−	−	−	−	−	40	12
	−	−	−	−	−	10	8
	−	−	−	−	+	10	9
	−	−	+	+	+	10	10
	+	+	+	+	+	10	3
	+	+	+	+	+	10	0

*20 mice per treatment, each given 2 ml. water or PABA solution (5 mg. per ml.) on the days prior to challenge as indicated: + = PABA, − = water.

would then be satisfied not by the host but by the metabolic products of the mutants themselves.

A nutritionally inadequate environment for the parasite may become a nutritionally adequate environment by altering the host's diet. Bacon *et al.* (1951) have provided an interesting model relating host diet to the virulence of a mutant of *S. typhosa* requiring PABA. Mice were fed PABA in their diet for a specific number of days prior to an intraperitoneal inoculation either with the mutant requiring PABA or the parental strain not requiring exogenous PABA. This biochemical mutant usually displaying a greatly diminished virulence became increasingly virulent for mice getting PABA in their diet prior to inoculation. Supplementing the diet with PABA prior to inoculation with the parental strain had no significant effect on the virulence of the parental strain (Table 4).

It might seem that studies on the virulence of biochemical mutants of pathogenic microorganisms represent laboratory models without parallel in nature. Formal *et al.* (1954) have documented a case of greatly reduced virulence in *S. typhosa* which was the result of a spontaneous mutation *in vitro* to a requirement for purine. Furthermore, it is now clearly established that the virulence of certain species of Plasmodium is related to the availability of PABA and methionine in the host (reviewed by McKee, 1951). For example, the growth and proliferation of certain species of Plasmodium is retarded *in vivo* in animals which have been starved either during a malarial infection or at the time of infection. This situation is maintained as long as the host remains starved. Growth and proliferation of the plasmodia follow the restoration of an adequate diet or the addition of methionine and/or PABA to the diet.

INHIBITION MECHANISMS

Perhaps the most common and important situation resulting in avirulence concerns the host which presents an adequate nutritional environment and an effective inhibitory environment. Inhibitory mechanisms may include biological, chemical, and physical antimicrobial agents. An appreciation of the significance of an effective inhibitory environment in the control of animal diseases resulted in the development of immunology. The most familiar biological antimicrobial agent is the phagocyte. It may often be difficult to distinguish between a physical and a chemical antimicrobial agent since a physical antimicrobial agent may act through a chemical antimicrobial agent. Antibodies and antitoxins represent familiar chemical antimicrobial agents. Although antibodies have not been conclusively demonstrated in plants, relatively simple compounds produced by certain resistant plant varieties have been found to be chemical antimicrobial agents (reviewed by Walker and Stahmann, 1955). Chemotherapy and vaccination represent an effort to create an effective inhibitory environment in a host presenting an adequate nutritional environment but an ineffective inhibitory environment, *i.e.*, a susceptible host. Temperature may be considered to be a physical antimicrobial agent.

To demonstrate an adequate nutritional environment and an effective inhibitory environment in a host may require special techniques adapted to the specific host and parasite. If a host species includes susceptible and resistant strains or varieties, it may be possible to correlate resistance with a specific inhibitory mechanism present in the resistant strains and absent in the susceptible strains. Although it would be desirable to isolate the mechanism for an *in vitro* demonstration, such a test may not always be possible. For example, Gothoskar *et al.* (1955) correlated resistance to Fusarium in the Jefferson variety of the tomato with the production of an antimicrobial agent; it was not possible to extract the presumed responsible chemical(s). However, the high resistance of onion varieties with pigmented bulb scales to the onion smudge fungus has been correlated with the presence

of protocatechuic acid and catechol (and not found in onion varieties with non-pigmented bulb scales) which act as chemical antimicrobial agents. This fascinating situation is reviewed in some detail by Walker and Stahmann (1955). Rich (1923) observed a similar situation in guinea pigs. A strain was found to be "free" of complement compared with other guinea pigs which had the normal high complement titer. The difference was controlled by a single gene, complement-less being recessive. Guinea pigs lacking complement were highly susceptible to *P. suiseptica* and *S. cholerae suis* when infected either experimentally or spontaneously. Morality in complement-less guinea pigs was 77 per cent; mortality in normal guinea pigs was 20 per cent.

THE PARASITE POPULATION

While the nutrition-inhibition hypothesis of pathogenicity emphasizes the host environment, it also assumes that the parasite remains unaltered in its characteristics. Such an assumption is valid only if it is supported by experimental evidence. Populations of parasites are relatively enormous so that mutants readily occur. Should an avirulent strain be able to proliferate to some degree in the host, it may be possible for a virulent mutant to arise and eventually to produce a population of virulent cells. In such an event, an avirulent strain may seem to have become virulent or a resistant host may seem to have become susceptible. Nutritionally competent mutants from nutritionally deficient strains would be able to proliferate or to metabolize in nutritional environments unsuited for the parental strain, *e.g.*, avirulent purine-requiring *K. pneumoniae* yielding virulent, purine independent mutants (Table 1). Mutants resistant to antimicrobial agents formerly effective for the parental strain would be virulent for formerly "resistant" hosts. For example, Garber *et al.* (1953) observed that *P. pestis* was extremely sensitive to streptomycin both *in vitro* and *in vivo*. Streptomycin therapy effective for mice infected with the parental sensitive, virulent strain was ineffective for mice infected with the mutant, streptomycin resistant strain. In this example, an ineffective inhibitory environment in the mouse for *P. pestis* was transformed into an effective inhibitory environment by the introduction of streptomycin. Consequently, the host could then be considered to be resistant to this pathogen. The occurrence of streptomycin resistant mutants of the pathogen would nullify the inhibitory effect of streptomycin. The role of mutation and selection in altering and maintaining populations of pathogens both *in vitro* and *in vivo* has been presented in considerable detail by Braun (1953).

The antigenic, morphological, and enzymatic characteristics of parasites are genetically controlled and not, at least directly, influenced by the host environment. For example, the ability to produce an enzyme is a genetic property of the parasite but the host may have to provide the necessary substrate. These characteristics of the parasite undoubtedly play an im-

portant role in the virulence of the parasite. While certain antigenic and morphological characteristics may represent a defense against certain inhibitory mechanisms of the host, other antigenic and morphological characteristics may not be significant. It may be important to establish criteria distinguishing these possibilities.

Burrows (1955) has presented an illuminating analysis of the basis of virulence for mice of *P. pestis* with relation to our current knowledge of the antigenic and morphological characteristics of this pathogen. "(phagocytosis) Resistant virulent organisms may have no observable capsule, but heavily capsulated virulent organisms are also phagocytosis-resistant, and very much more so than are avirulent cells capsulated to the same degree. It is probable that capsulated virulent organisms are resistant for the same reasons that non-capsulated virulent organisms are resistant—a situation which is understandable by assuming the presence of an invisible, but nevertheless effective, degree of capsulation in the latter. If this is so it must be presumed that the capsular material of virulent organisms has, *in its native state*, some different properties from that of avirulent organisms, despite the fact that extracted products from virulent and avirulent organisms apparently have identical properties. It is not unprecedented in immunology for biological materials to differ in physiological activity although serologically indistinguishable." (Burrows, 1955)

The probable role of enzymes has been succinctly presented by Dubos (1954). "All the microbial enzymes that we have just briefly considered take a direct or indirect part in pathological processes, but—except for the lecithinases of clostridia—their effects are difficult to evaluate because they do not cause obvious toxic reactions. It is important to notice that they do not kill the tissue cells which they attack and that some of them (for example, hyaluronidase, collagenase, dornase, fibrinolysin) appear to hydrolyze only substrate present in the extracellular environment. As a consequence, their toxic effects are only those of the split products of their specific substrates and are not apparent unless large amounts of enzymes are involved. It is probable that they affect the course of infection only indirectly by changing the physical and chemical characteristics of the environment surrounding the parasite."

It may be profitable to consider pathogenicity as a consequence of a series of events. First, the parasite must proliferate from the relatively small population in the "normal" inoculum to a characteristically large population. Second, the parasite must be able to produce the necessary materials (enzymes?) to alter the immediate environment in the host if such an alteration is required. Third, the parasite must be able to affect the host *in toto* or specific cells or tissues of the host by producing specific materials (enzymes, toxins, antimetabolites, *etc.*). Failure to accomplish any one of these steps may result in avirulence. A study of "the biochemical determinants of microbial diseases" (Dubos, 1954) may yield more far-reaching information than has yet become available from current techniques.

SUMMARY

A nutrition-inhibition hypothesis of pathogenicity is proposed on the basis of an ecological point of view. This hypothesis considers the two environments in the host which directly affect the fate of an invading parasite: a nutritional environment and an inhibitory environment. The nutritional environment may be adequate or inadequate to support a proliferating or metabolizing population of the parasite; the inhibitory environment may be effective or ineffective in suppressing the proliferation or metabolism of a population of the parasite. Of the four possible combinations, only the adequate nutrition-ineffective inhibitory environment results in virulence. Examples of virulence and avirulence as a consequence of the parasite encountering specific host environments are presented. The possible role of the antigenic, morphological, and enzymatic characteristics of the parasite as they relate to pathogenicity is discussed.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

MODES OF TETRAD FORMATION

A fundamental concept in the theory of genetic linkage is that of the mode of formation of the meiotic products, whether these be tetrads or single gametes. In the study of the gametic output of diploids this idea is a fairly familiar one. A diploid organism heterozygous at two linked loci may produce gametes of four different genotypes but there are only two different modes of formation, corresponding with the presence or absence of recombination between the two loci. In general, a diploid organism which is heterozygous at any number n of linked loci may produce gametes of 2^n different genotypes but there are only 2^{n-1} different modes of gamete formation, corresponding with the presence or absence of recombination in each of the $n-1$ intervals. Any mode of formation can lead to gametes of two different genotypes which are determined by the parental genotype.

With tetrads, there are not only many more genotypes and modes of formation than there are for the gametic output but the ratio of the number of tetrad genotypes to the number of modes of formation depends on the number of loci considered. With a given number of loci, some modes of formation lead to one and others to two tetrad genotypes.

Ordered Tetrads. In an ordered tetrad, the two pairs of products of the first meiotic division can be distinguished and this distinction must be shown in the genotype of the tetrad. If W, X, Y and Z represent the four products of a single meiosis in which W and X separated from Y and Z at the first division, the genotype of this ordered tetrad may be written in the form

W, X ; Y, Z.

No significance is to be attached to the order of arrangement within either pair or between the two pairs of products and the given genotype therefore could also have been written in any of the following equivalent forms:

	Y, Z ; W, X
W, X ; Z, Y	Z, Y ; W, X
X, W ; Y, Z	Y, Z ; X, W
X, W ; Z, Y	Z, Y ; X, W

With respect to a single gene difference A-a, there are two possible genotypes for an ordered tetrad:

A, A ; a, a and A, a ; A, a.

A diploid parent Aa produces tetrads of these two genotypes by two different modes of formation corresponding to reductional and equational separation respectively. The relative frequencies of occurrence of these two modes of formation are characteristic of the particular locus in question and must be determined experimentally.

A diploid organism heterozygous at two linked loci may give rise to ordered tetrads of seven different genotypes. If B and b denote the genes available at the second locus, these genotypes are as follows:

AB, AB ; ab, ab
Ab, Ab ; aB, aB
AB, aB ; Ab, ab
AB, Ab ; aB, ab
AB, ab ; AB, ab
AB, ab ; Ab, aB
Ab, aB ; Ab, aB .

Since every one of these genotypes is unaltered by an interchange of parental chromosomes (whether these be AB and ab, or Ab and aB), each one may be said to correspond with a different mode of tetrad formation. The specification of the output of tetrads from a parent heterozygous at two linked loci requires knowledge of the frequencies of occurrence of these seven modes of formation. These frequencies, which are characteristic of the pair of loci concerned, are not given by theory but must be determined experimentally from tetrad analysis.

With three or more linked loci, there are more tetrad genotypes than modes of tetrad formation. Some pairs of genotypes correspond with the same mode of formation. Whitehouse (1942) has demonstrated that there are 32

TABLE 1

THE 32 GENOTYPES OF ORDERED TETRADS WITH SEGREGATION AT THREE LINKED LOCI. (AFTER WHITEHOUSE, 1942)

i) AA aa

				1	C c	C c	C c
				2	C c	c C	c C
				3	c C	C c	c C
1	2	3	4		c C	c C	C c
B	B	b	b		1 2	5	
b	b	B	B		3 4	6	
B	b	B	b			9 10	11 12
b	B	b	B		7 8	10 9	12 11
B	b	b	B			11 12	9 10
b	B	B	b			12 11	10 9

ii) Aa Aa

				1	C c	C c	C c
				2	C c	c C	c C
				3	c C	C c	c C
1	2	3	4		c C	c C	C c
B	B	b	b		13 14	15	16	17 18
b	b	B	B		14 13			18 17
B	b	B	b		19	21	22	25
b	B	b	B		20	23	24	26
B	b	b	B		27 28	29	30	31 32
b	B	B	b		28 27			32 31

different "classes of arrangement" of the four products in the case of three linked loci and 172 for four loci. These are in fact the different genotypex. For three linked loci they are shown in table 1 (cf. Whitehouse *loc. cit.* Table 3). Fisher (1950) has determined the number of modes of formation of ordered tetrads with any number of linked loci and in particular, has shown that there are 29 and 136 modes for the cases of three and four linked loci respectively. With three linked loci, there are three fewer modes of formation than there are genotypes because six of the 32 tetrad genotypes can be arranged into three pairs, the two genotypes of a pair corresponding with the same mode of formation, being obtainable from one another by a simple interchange of the parental chromosomes. For example, when the parental genotype is ABC/abc, these three pairs of genotypes are

ABC, Abc ; aBc, abC and abc, aBC ; AbC, ABc,
ABC, aBc ; abC, Abc and abc, AbC ; ABc, aBC,
ABC, abC ; Abc, aBc and abc, ABc ; aBC, AbC,

i.e., those numbered 11 and 12, 17 and 18, and 27 and 28 respectively in table 1. The two tetrads of any of these three pairs will have the same expected frequency of occurrence. The frequencies with which tetrads are produced by the 29 different modes of formation will be characteristic of the particular set of three loci concerned and must be determined experimentally.

Fisher (1950) showed that the number of modes of formation of ordered tetrads with n linked loci is

$$(6^n + 3.4^n + 7.2^n)/16,$$

$$2, 7, 29, 136, 692, 3712, \ldots$$

The number of tetrad genotypes, which can be obtained very readily from Fisher's analysis on neglecting interchanges between homologous genes (Aa), is

$$(6^n + 5.2^n)/8,$$

$$2, 7, 32, 172, 992, 5872, \ldots$$

Hence, as the number of linked loci is increased, the number of tetrad genotypes increases relative to the number of modes of formation until almost every mode of formation is able to give rise to tetrads of two different genotypes. It should be noted that Whitehouse (1942) gave

$$\frac{3}{2}(n + 1)! - 2^{n-1},$$

$$2, 7, 32, 172, 1064, 7528, \ldots$$

as the number of classes into which ordered tetrads could be grouped, while according to Papazian (1952), 'the number of classes of ordered tetrads, pooling only those classes that give no information as to crossing-

over is $(5.2^n + 6^n)/8$.' Whitehouse's formula is incorrect while Papazian has enumerated, not the classes to which he refers, but tetrad genotypes.

Whitehouse (1942) and also Papazian (1951) have distinguished two kinds of three-strand double crossovers. This is equivalent genetically to distinguishing between the two tetrads of a pair produced by the same mode of formation, a distinction that is not necessary for the genetic analysis of linkage in tetrad material.

Unordered Tetrads. In an unordered tetrad, the products of the first meiotic division cannot be distinguished. Hence the genotype of an unordered tetrad in which the four meiotic products are W, X, Y and Z, may be written in the form W, X, Y, Z where no attention is paid to the order of arrangement. An unordered tetrad which has been produced by the diploid heterozygote Aa must have the genotype A, A, a, a. With two linked loci, there are three possible genotypes:

AB, AB, ab, ab,
Ab, Ab, aB, aB,
and AB, Ab, aB, ab.

Since each of these is unaltered by interchange of the parental chromosomes, there must be three modes of tetrad formation in this case. If the parental genotype is AB/ab, the tetrad genotypes given above may be described as parental ditype (P), recombinant ditype (R) and tetratype (T) respectively. These descriptions specify the mode of formation.

With three linked loci, there are twelve possible genotypes for an unordered tetrad but only eleven modes of formation. The twelve genotypes are in

TABLE 2

THE 12 GENOTYPES OF UNORDERED TETRADS WITH SEGREGATION AT THREE LINKED LOCI, CLASSIFIED ACCORDING TO THE 11 MODES OF FORMATION BY DISTINGUISHING THE THREE PAIRS OF LOCI AS PARENTAL DITYPE (P), RECOMBINANT DITYPE (R) OR TETRATYPE (T). THE ORDER IN WHICH THE THREE PAIRS OF LOCI ARE DISTINGUISHED IS A-B, B-C, A-C. (COMPARE WITH TABLE 1(i))

AAaa

					P	N	T			
				1	C	c	C	c	C	c
				2	C	c	c	C	c	C
				3	c	C	C	c	c	C
	1	2	3	4	c	C	c	C	C	c
P	B	B	b	b	PPP	PNN	PTT			
N	b	b	B	B	NNP	NPN	NTT			
T	B	b	B	b	TTP	TTN	TPT	TNT	TTT	
	b	B	b	B			TNT	TPT		
	B	b	b	B			TTT		TPT	TNT
	b	B	B	b					TNT	TPT

fact those shown in table 1(i). The two genotypes numbered 11 and 12 correspond with the same mode of formation. The eleven modes of formation of unordered tetrads can be distinguished by classifying the three pairs of loci as P, R or T (see table 2).

Papazian (1952) has suggested that with unordered tetrads there is a loss of information when, instead of giving the genotypes of the tetrads, all pairs of loci are classified as P, R or T. With unordered tetrads, however, classification of all pairs of loci as P, R or T is equivalent to classification according to the mode of formation and this is all that is needed for genetic analysis.

Fisher (1950) showed that with n linked loci, the number of modes of formation of unordered tetrads is

$$(6^n + 3.4^n + 15.2^n)/48,$$

$$1, 3, 11, 48, 236, 1248, \ldots$$

The number of different possible genotypes for unordered tetrads with n linked loci is

$$(6^n + 9.2^n)/24,$$

$$1, 3, 12, 60, 336, 1968, \ldots$$

As with ordered tetrads, when the number of linked loci is increased, the number of genotypes of unordered tetrads increases relative to the number of modes of formation until almost every mode of formation can give rise to tetrads of two different genotypes.

SUMMARY

The classification of the genotypes of ordered and unordered tetrads according to their mode of formation is discussed and it is shown that as the number of linked loci is increased, almost every mode of formation is able to give rise to tetrads of two different genotypes.

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LETHALITY INDUCED BY FEEDING RADIOPHOSPHORUS TO MALE HABROBRACON

Inquires about the offspring from adult male wasps fed radioisotopes have been received both from geneticists interested in mutation and from health physicists concerned about the ecological influence of radioactive contaminants. Results available in scientific journals are either from experiments with adult females (Grosch and Sullivan, 1952, 1954a) or from larval feeding (Grosch and Sullivan, 1954b). In 1952, six months were devoted to feeding p^{32} adult male Habrobracon but this type of investigation proved to be an uneconomical expenditure of time, effort and funds. In the first place, males cannot undergo starvation as well as females (Grosch, 1950). Many become enfeebled and a few die during the necessary starvation period preliminary to feeding. Those surviving are not persistent in their mating attempts. Furthermore, even though copulations are observed, a low frequency of biparental offspring with an accompanying increase in haploid males often indicates unsatisfactory mating or lowered sperm supply even in control wasps. In general, males held the necessary lenght of time show signs of senility.

Results from the best experiment out of the many attempted are shown in table I. These are calculated on the basis of offspring per mated female. A group of 20 starved males from stock 17 were fed honey adulterated with p^{32} at a concentration of 200 uc/gm of mixture. One day after feeding, the males were mated in an outcross to virgins isolated from stock 33. In crosses of unrelated stocks, all females come from fertilized eggs, all males from unfertilized. The appearance among the offspring of an appreciable number of females, the biparental sex, indicates that the treated males were far from sterile. Additional matings by a few males still alive two days after the first mating gave similar results (not shown).

The more important aspect of the data in table I is the depression in numbers of female offspring without an accompanying increase in sons from treated fathers. As established by P. W. Whiting and associates, this provides a measure of the induction of dominant lethals. Only in a naturally parthenogenetic species can the phenomenon easily be separated from male sterility due to inactivation or failure in production of spermatozoa. In the latter cases the number of males would be increased.

For the present experimental offspring, a sex ratio of nearly 1:1 was obtained instead of the typical 60 to 70% predominance of females. When table I is compared with Heidenthal's 1945 x-ray results, the level fed is biologically equivalent to not more than 2000r. If fed to females, the same mixture can cause permanent cessation of egg production, an effect requiring 5000r of x-rays (Grosch and Sullivan, 1954a). The lowered egg hatchability caused by 2000r of x-rays can be mimicked by feeding females mixtures containing only 100 μc/gm of p^{32}. Thus even when successful, radio-

TABLE 1

A COMPARISON WITH CONTROL DATA OF THE F 1 FROM MALES FED FROM P^{32}

One day after feeding		Eggs/ Mother	Per Cent Survival to Adults	Adults/Mother			Total ♀♀/ Total Progeny
				Female	Male	χ^2	
First Mating	T	236.91	46.15	58.09	51.24	4.25	0.53
	C	214.33	56.60	76.02	45.30		0.63
Second Mating	T	204.24	43.90	45.28	44.39	3.59	0.51
	C	233.87	55.03	77.60	51.10		0.60
Combined Total	T	441.15	45.11	103.37	95.63		0.52
	C	448.20	55.78	153.62	96.40		0.61

T = treated
C = control

phosphorus feeding experiments show considerably less effect when males rather than females are used.

In order to corroborate evidence of dominant lethality, the cocoons holding uneclosed pupae were opened and the contents identified if possible. Nearly twice as many unemerged females were obtained from treated fathers as from controls, but as expected, the number of unemerged sons found as dead pupae was identical in control and experimental fraternities. Furthermore there was a great increase in unidentifiable prepupae among offspring from p^{32}-fed males representing wasps which had died shortly after spinning. Presumably even more biparental offspring die as embryos. In one specific check of 100 offspring, 87% of those dying did so as embryos.

Studies appearing subsequent to our experiments have suggested an explanation of the relative ineffectiveness of radiophosphorus fed Habrobracon males. Autoradiographs show no radioactivity concentrated in the testis, in contrast to the female where the gonads are a main route of isotope disposal (Grosch and Sullivan, 1953). Furthermore, Pelc and Howard (1953) reported a very slow turnover for DNA phosphorus, and King (1953) emphasized that synthesis of new DNA is a foremost requirement in the mutagenic functioning of recoil and valence changes. Synthesis is not occurring in the testes of adult Habrobracon males since Henschen (1928) showed spermatogenesis to be largely completed by the time of eclosion. Therefore, heritable changes, if any, are induced by emanations from the soma and the gut contents. Finally, since in natural conditions braconid males will rather mate than eat, the ecological influence on the braconid species of ingested radioactive material should be relatively slight.

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ADAPTIVE AND SELECTIVE VALUE

We are glad that Li (1955) has entirely confirmed our conclusions (1954) as to the uselessness of w in comparing populations, and of selection coefficients of alternatives within a population for estimating the adaptiveness of populations homozygous for one alternative. Nevertheless, he asserts that we have deeply misunderstood the analytical method employed in gene frequency analysis. However, his comments clearly indicate that he has himself misunderstood our paper. He agrees that a standardisation of terms and avoidance of loose language is desirable, but overlooks the fact that it is *not* true that one word is as good as another if precisely defined; that depends on the accepted usages of the words in question. No one is going to say that we may as well call men fruitflies if the terms are defined precisely enough; and one of our points is that adaptation has had on the vast majority of occasions a useful meaning quite different from that of selection, that the use of adaptive value for selective value (or selection coefficient, or its derivatives) now leads to serious confusion, and that it is precisely this confusion that has led Dobzhansky to put forward the arguments which we have criticised, in which criticism Li himself supports us.

Li's misunderstanding is clearly seen in his treatment of the isolated sentence which he quotes from our paper and decorates with wrongly-placed italics. If italics are necessary, they should be used for the word "seems". We are commenting in this sentence that Dobzhansky, by using the word

adaptive where selective is appropriate, *seems* (we do not wish to be dogmatic) to believe that populations with a high value of $\bar{w}$ are necessarily more highly adapted in the usual sense of the word than those with a low value. This comment seems justified because Dobzhansky (1951, p. 123) does use selection coefficients of alternatives within a population to draw conclusions as to homozygous populations being at a disadvantage, which only makes sense if he considers that these selection coefficients do necessarily give information on the *adaptedness* to a given environment of the alternatives taken separately. We are disagreeing with this view.

A similar misunderstanding is the basis of Li's remark that our "grasp of the intra-population selection concept is somewhat incomplete" on the grounds that no selection coefficient ever gives information on the ability to survive of homozygous populations, whereas we have said that "a particular homozygote may be at an extreme disadvantage because of extensive dislocations of its structure and physiology that result from homozygosity, irrespective of what genotypes may be coexisting with it in the same population. In this situation the selection coefficient relative to other genotypes does give a rough measure of the homozygote's chances of survival in pure cultures." The example given by Li to disprove this assertion proves nothing either way; the sort of disability we were referring to was failure to hatch from the egg at all, *irrespective of what other genotypes may be in the population.* But, as we point out immediately after the quotation given above, a mere inspection of selective values does not allow one to recognise such a situation. What is necessary is both the selective value and information on the effects of the gene or inversion in question.

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No undertaking to publish reviews is implied in acknowledgement of publications received. Books for notice may be sent to:

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Aubert de la Rue, E., 1955. Man and the winds. 206 p., ill. $6.00. Philosophical Library, New York.

Barker, Robert G., and Herbert F. Wright, 1955. Midwest and its children. 532 p. Row, Peterson and Company, Evanston Illinois.

Beebe, William, 1955. Adventuring with Beebe. 283 p., ill. $4.50. Duell, Sloan & Pearce, New York. Little, Brown, Boston.

Selections from the writings of William Beebe, now Director Emeritus of the Department of Tropical Research, New York Zoological Society. Among the varied essays from the pen of this well-known naturalist is included an absorbing account of the first descent in the bathysphere with Otis Barton.

Berrill, N. J. 1955. Man's emerging mind. 308 p. $4.00. Dodd, Mead and Company, New York.

Future historians of the present era may well marvel at the wholesale application of scientific logic to the diversified areas of research, each of which is influenced by changing theoretical, practical and emotional interests. They will, without doubt, pay heed to the sum of high intelligence and to the eagerness of the individual scientist to produce results, and they will hardly underestimate achievements such as the unifying theories in the physical sciences, the reconnaissance of organic and ecological systems in biology, and the plans of psycho-sociologists to solve problems by concerted and prolonged analysis of human personality and culture. They finally may record earnest efforts by scientists of coming more constructively to grips with the cultural and social consequences of scientific invention.

Professor Berrill has chosen as his topic the evolution of *Homo sapiens*, against the background of organic, environmental and cultural change. He has succeeded remarkably well in reconstituting the man-nature event which now lies buried in the past, and it is not solely his simple, lucid and frequently humorous style which evokes a feeling of cosmic proportions worthy of that dramatic event. Berrill makes quite clear that man has arrived at a turning point where he must choose either

to take stock of his natural resources and of his potentialities for humanized living, or perish.

This dilemma is not so obvious in the description of the early phases of human development. Here one learns, among other things, about dating rocks and bones, evolution from a small placental mammal toward ape and man-ape, early and much later "explosive" brain evolution, nakedness, tool-making, use of fire, burial rites, ancestor worship, changing art styles among stone age men, and expressions of primitive man's reverence for life. Persistence of primitive modes of behavior as well as man's most characteristic development, the "Prolongation of Youthfulness," are discussed. There are sections which deal with history and cultural change in the more modern sense, for example, with neolithic cultures, domestication, invention of the polished ax and the bow, irrigation farming, pottery wheel, bronze age civilization, the flood, Mesopotamian culture, and the still existing Hopi society. Gradually, however, the condition of man becomes more precarious on a different scale. His earlier destiny had been shaped to a large extent by the ever-changing climate and by population pressures; now the globe is filled, populations are outgrowing their food supply, and the balance of nature has changed from the many and manifold to the all-consuming one. Man's striving for adventure and freedom has remained the same nevertheless, and he may be forced to re-organize the direction in which he employs his energies. It would be wise to live within the natural budget since on this earth technological miracles have their limits and emigration to other planets remains doubtful. Man's chief difficulty now is certainly his remoteness to actual conditions and his helplessness in the face of simple creative necessities. Symptoms of man's present impotence are the dangers of atomic experimentation and extermination, the general technical hypertrophy of our time, and instances of cheap salesmanship in science. Professor Berrill is not certain that in our present society any future discovery, such as the possible control of human sex, would not be deliberately exploited to serve particular ends.

But this book has a very positive side, namely confidence in the mind and capacity of the average individual not deafened by propaganda, and it looks forward to a lofty and enlightened science that knows how to respect the laws of organic growth, for "the two most obvious characteristics of nature are loveliness and power, and when we concentrate upon power we do so at our own peril." The reader will be grateful for philosophical digressions which state the author's faith in an all-pervading unity in nature and a correspondence between the ways of nature and our own perceptive capacities. He believes that every individual life attains value and meaning in both past and future. "There is richness here if only we will give ourselves to being alive and cease struggling to disown our nature . . . . It is high time we listen."

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